VOTING FOR BETTER HEALTH IN DEPRIVED COMMUNITIES

Quine, Anna; Obuzor, Gladys U.; Nthula, Nthusi; Ndomoto, Lilian; Tulinius, Charlotte; Hibble, Arthur; Chahal, Prit; Nyanja, Njeri; Barasa, Sammy

Published in:
American Journal of Tropical Medicine and Hygiene

DOI:
10.4269/ajtmh.abstract2016

Publication date:
2017

Document version
Publisher's PDF, also known as Version of record

ASTMH
AMERICAN SOCIETY OF TROPICAL MEDICINE & HYGIENE
ADVANCING GLOBAL HEALTH SINCE 1903

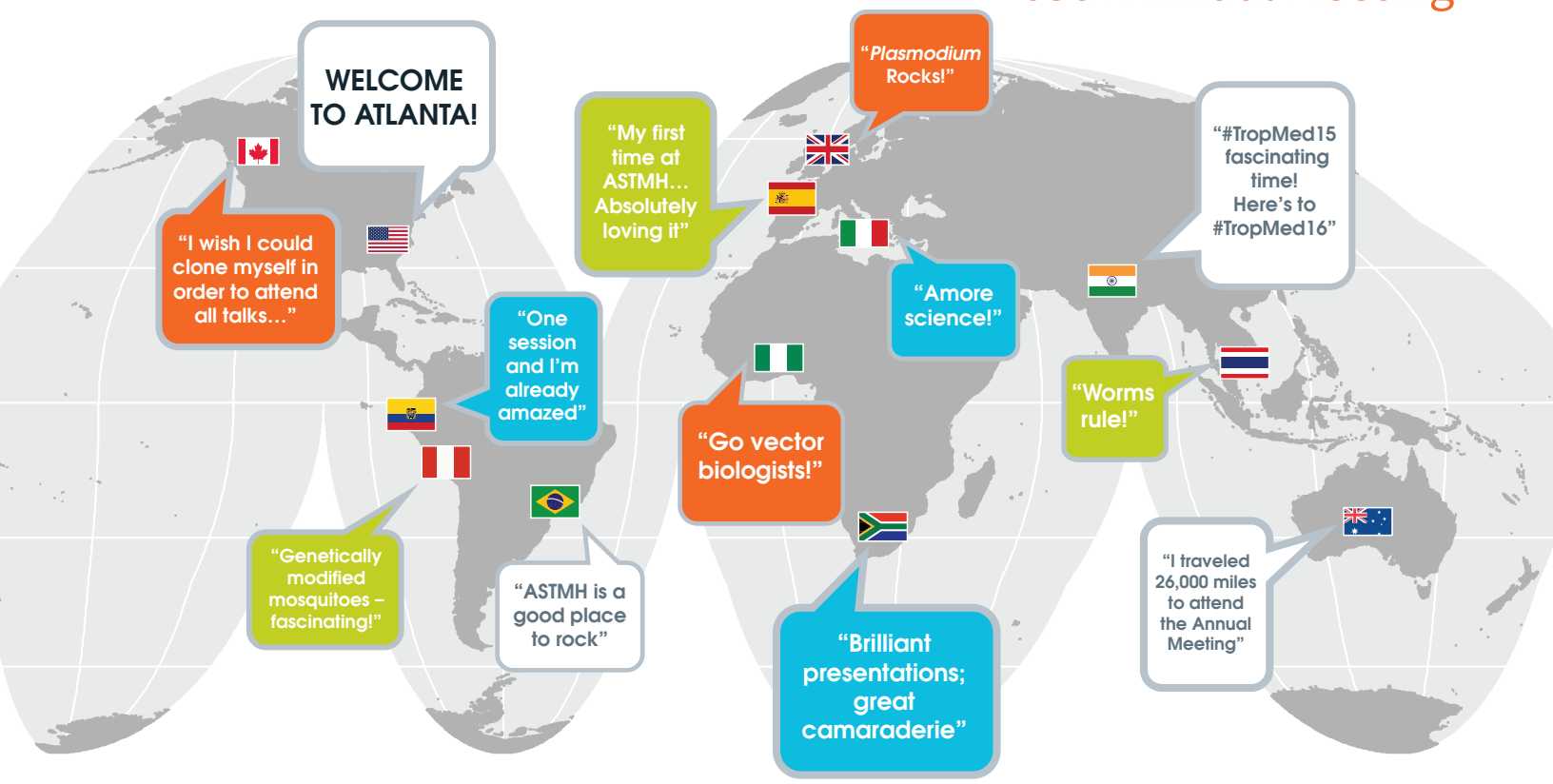
VOLUME 95 NOVEMBER 2016 NUMBER 5 SUPPLEMENT

Abstract Book

November 13–17, 2016

Atlanta Marriott Marquis
Atlanta, Georgia USA

65th Annual Meeting



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Supplement to
The American Journal of Tropical Medicine and Hygiene

1

LYMPHATIC FILARIASIS RESIDUAL TRANSMISSION HOTSPOTS IN AMERICAN SAMOA

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To achieve elimination of lymphatic filariasis, American Samoa carried out 7 rounds of mass drug administration from 2000 to 2006 and passed Transmission Assessment Surveys (TAS) in 6-7 yr-old children in 2011 and 2015, although 1 or 2 ICT-positive children were found on each occasion in the same school. Serology studies using Og4C3, Wb123 and Bm14 in 807 adults in 2010 identified higher antigen prevalence in two spatial clusters, one of which included the school attended by ICT-positive children in both TAS. Prevalence was higher in men and those residing in American Samoa for <5 yrs. To follow up these findings, a targeted study in 2014 tested three groups of individuals: 124 residents aged 3-70 yrs in the two putative 'hotspots'; 337 children aged 7-13 yrs in the school where ICT-positives had been identified in TAS; and 650 adult workers (residing across the island) attending a pre-employment clinic or working in the tuna cannery. Overall prevalence (N=898 to 1,111 depending on the test) was 2.1% (95% CI 1.3-3.1%) for ICT and Og4C3, 5.7% (4.2-7.3%) for Wb123, and 10.2% (8.5-12.2%) for Bm14. The study confirmed elevated prevalence of ICT and Og4C3 (both 8.1%) antigen as well as Wb123 (9.8%) and Bm14 (23.6%) antibody in all ages in the two suspected hotspots. Bm14 antibody prevalence was higher in males than females in all groups (32.1% vs 17.1% in hotspot villages (p=0.05); 19.9% vs 7.0% in adult workers (p<0.001); and 3.3% vs 0.6% in children aged 7-13 yrs (p=0.06)). All ICT positive persons were treated and had slides taken. Microfilariae (Mf) with density from 8 to 3267/ml were observed on 4 of 20 slides examined, with all Mf positive persons residing in hotspots. Thus age, gender and residence in a hotspot village were the predominant risk factors for being positive for diagnostic markers of LF. This study has confirmed the suspected hotspots previously identified in 2010 from a spatially representative adult sample as sites of continuing transmission and potential sources of resurgence. The results further support the potential use of spatial epidemiological methods for identifying residual foci of infection in the endgame phase of LF elimination programs.

2

EXCESS MORTALITY ASSOCIATED WITH HIGH *LOA LOA* MICROFILAREMIA IN THE EAST REGION OF CAMEROON: A RETROSPECTIVE COHORT STUDY

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Loiasis affects more than 10 million people, most in Central Africa. Besides its classical signs ("eye worm" and "Calabar swelling"), it has also been reported as a cause of renal and cardiac complications. However, the burden of loiasis has never been assessed and it is still considered a benign condition. To assess whether loiasis bears any excess mortality, we conducted a retrospective cohort study in the East Region of Cameroon. In 2001, 3,627 individuals living in 28 villages were included in a survey during which thick blood smears (50 µL) were analyzed for *Loa loa* microfilarial (mf) density. In 2016, these villages (where no mass ivermectin treatment has ever been organized) were visited again to assess whether the subjects examined 15 years before were still alive. The vital status could be determined for 3,301 individuals (91%). Data analyses included (a) an analysis at the community level between the age- and sex-standardized prevalence of (hyper)-microfilaremia in 2001 and the standardized mortality rates (SMR); (b) an assessment, using multivariate accelerated failure model, of the excess mortality relative to the initial mf density (4 classes: 0, 1-8,000, 8,000-30,000 and >30,000 mf/mL); (c) the calculation of the population-attributable fraction of mortality due to presence (vs. absence) of a Loa microfilaremia. At community level, the SMRs increased by 5.5% when the proportion of subjects with >30,000 mf/ml increased by 1% (P=0.040). A similar trend was observed when the threshold was 8,000 mf/ml (2.9%/1%; P=0.068). People aged >25 years with more than 30,000 mf/mL in 2001 died significantly earlier than those with lower mf densities (Time Ratio=0.67, 95% CI: 0.48-0.95, P=0.024). Lastly, 14.5% (95% CI: 6.5-21.8) of all-cause mortality was attributable to the presence of *Loa* mf. In conclusion, high *Loa* microfilaremia was associated with increased mortality in the study site. There is a need to validate our observations in other Loa areas, as they are likely to have implications on the status of loiasis in terms of public health, and the implementation of onchocerciasis and lymphatic filariasis elimination programs in Central Africa.

3

PROGRESS TOWARDS ONCHOCERCIASIS ELIMINATION IN THE PARTICIPATING COUNTRIES OF THE AFRICAN PROGRAM FOR ONCHOCERCIASIS CONTROL: EPIDEMIOLOGICAL EVALUATION RESULTS

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The African Programme for Onchocerciasis Control (APOC) was created in 1995 in order to control onchocerciasis as a public health problem by implementing sustainable Community Directed Treatment with Ivermectin (CDTI). When research showed that mass treatment can lead to complete elimination of the infection in Africa, APOC shifted its target to elimination of infection and interruption of transmission. Epidemiological evaluations have been undertaken from 2008 to 2014 in evaluation areas with ≥6 years of effective treatment. We analyzed these unique data, to assess progress towards elimination. Epidemiological evaluations were done per area, in two phases. First, parasitological surveys were done in about 10 selected high risk communities per area with high pre-control endemicity level. By comparing observed prevalence levels to expected trends (as predicted by the established ONCHOSIM model, developed at Erasmus MC Rotterdam, and extensively used for policy support in onchocerciasis control in Africa), using Bayesian methods and Monte Carlo simulation, we classified the progress towards elimination as "faster than predicted", "on track", or "delayed". Second, in areas close to elimination, additional parasitological surveys were done in more communities to assess whether mass ivermectin treatment can safely be stopped. Initial parasitological surveys covered 54 areas, 639 villages and 127,665 people out of 53 million total population. The decline in prevalence was faster than predicted in 23 areas, on track in another 23 and delayed in 8 areas. Additional surveys were done in 22 areas and 13 of these met the epidemiological criteria for stopping treatment. Overall, 32 areas (25.4 million people) had reached or were close to elimination, 18 areas (17.4 million) were on track but required more years treatment, and in 8 areas (10.4 million) progress was unsatisfactory. Great progress has been made by APOC in realizing elimination beyond its prime goal. Elimination is reached or close for millions of people. Extra effort is needed in areas with unsatisfactory progress.

4

DETECTING INFECTION HOTSPOTS: MODELING THE SURVEILLANCE CHALLENGE FOR ELIMINATION OF LYMPHATIC FILARIASIS AND OTHER DISEASES

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Confirming elimination of lymphatic filariasis (LF) is a challenge due to its existence in small endemic foci (microfoci; µf). The capacity of post-treatment surveillance (PTS) to detect µf is unclear. We used microsimulation modeling to assess the ability of different types of PTS to detect LF µf in a background of low prevalence. Five µf of radius 1, 2, or 3 km with infection marker prevalence (intensity) of 3, 6, or 10 times background were placed in each simulation, run in R Version 3.2. Tests included microfilaremia, immunochromatographic test (ICT), and Wb123 ELISA. We set population size (360,000) and area (60 x 60 km) and based demographics on literature; background ICT prevalence in 6-7 year olds was 1.0%. Adults≥18 years, women 15-40 ('WCBA') years, 6-7 year olds, or children≤5 years were sampled. Cluster (CS) or simple random sampling (SRS) was conducted, with follow-up testing of nearest 20, 100, or 500 persons to each positive. A threshold count of positive persons in follow-up testing indicated a suspected µf. Suspected and true µf were compared for predictive value positive (PVP). Each parameter set was referred to as a protocol. Protocols were scored by efficiency, defined as the most µf identified and fewest persons tested. Of 3402 protocols, 384 (11.3%) identified all 5 µf (PVP 3.4-100.0%) testing 0.73-35.6% of the population. All used SRS; 378 (98.4%) only identified all 5 µf if they were 2-3 km diameter or intense infection (6x or 10x). 374 (97.4%) required ICT or Wb123 and 281 (73.0%) required sampling adults or WCBA. The most efficient CS protocol identified 2/5 µf. After limiting to 1-km radius µf with 3x intensity (n=378), 8 protocols identified all 5 µf; all used SRS and ICT but required testing 31.2-33.3% of the population. Nine protocols identified 4 µf, all using SRS and ICT with adults or WCBA and testing 3.5-9.7% of the population. Of those using CS (42.2%), only 14 (8.6%) identified any µf (1 of 5). Of the protocols using microfilaremia tests, only 6 (3.7%) identified any µf (1 of 5). In this model, SRS, ICT and sampling of adults maximized µf detection efficiency. The model provides many PTS protocols that can be selected for optimal outcomes.

5

USE OF AN ADULT TRANSMISSION ASSESSMENT SURVEY TO ASSESS PERSISTENCE OF LYMPHATIC FILARIASIS AT THE EVALUATION UNIT LEVEL IN GALLE, SRI LANKA

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Sri Lanka's Anti Filariasis Campaign (AFC) conducted 5 annual rounds of mass drug administration (MDA) with DEC plus albendazole in all endemic regions in the country between 2002-2006. Post-MDA surveillance has consistently documented microfilaremia (Mf) rates <1% in all sentinel and spot check sites, and all implementation units easily satisfied WHO transmission assessment survey (school-based TAS) targets in 2013. However, alternate surveillance methods documented persistence of LF in some areas. For example, molecular xenomonitoring (MX) surveys showed alarmingly high rates of filarial DNA in mosquitoes in the coastal Galle evaluation unit (EU) in southern Sri Lanka. The purpose of this study was to explore the utility and feasibility of AdultTAS for detecting low level persistence of *W. bancrofti* infection at the EU level using coastal Galle (population 0.7 million) as a positive control study site. We used Survey Sample Builder to randomly select two samples of 30 evaluation areas (EAs, mean population per EA 3,000), and approximately 1,800 adults were tested for filarial antigenemia with the Alere Filariasis Test Strip (FTS) in each of EA samples. Thirty of these EAs had previously been assessed for persistent LF by MX. The FTS rate for the entire study sample (N=3,612) was 1.8% (CI 1.4-2.2), and rates for the two sets of 30 EAs were 1.5% (CI 1.0-2.2) and 2.0% (CI 1.4-2.7), respectively. Thus the two EA samples provided similar results for the EU with upper CI values that exceeded the 2% target. FTS rates by EA were highly variable (range 0-11%) and exceeded 5% in 6 EAs. FTS rates in adults and filarial DNA rate in mosquitoes for 30 EAs tested by both methods were significantly correlated (r = 0.43; P=0.02). This study has shown that AdultTAS is more sensitive than school-based TAS and more convenient than MX for detecting persistent LF following MDA. Results from AdultTAS may be useful for identifying hot spot areas that require mop-up activities. AdultTAS is feasible for use by national LF elimination programs at the EU level, and it should also be more useful than school-based TAS for remapping "non-endemic" areas that were not included in MDA programs.

6

IMPACT OF LONG LASTING INSECTICIDE TREATED BEDNETS ON LYMPHATIC FILARIASIS PREVALENCE IN PAPUA NEW GUINEA

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Vector control efforts against malaria may accelerate lymphatic filariasis (LF) elimination in coendemic areas with anopheline LF transmission such as Papua New Guinea. Long lasting insecticide treated bednets (LLIN) were distributed nationwide in Papua New Guinea in 2009. Mathematical models have predicted that LLIN could be sufficient to end transmission in some of these study communities based on the previously described decrease in anopheline mosquito density and LF vectorial transmission potential, as reported previously. Here, we evaluate the impact on human markers of LF infection 5 years after LLIN distribution in the absence of mass drug administration (MDA) in communities previously shown to have different LF transmission levels. 1,262 Night-time finger prick blood samples were collected January-May, 2015. These samples were assessed for microfilaria (MF), ICT antigen card test, and BM14 antibody test. Current bednet usage was self-reported to be >80%. The moderate transmission communities had zero MF positive individuals and only one ICT-positive individual among children under 10 years of age (living most of their lives post-LLIN). Although age adjusted prevalence had decreased from 5.0% to 2.4% (p<0.001) during this time, older age groups were observed with mf prevalence up to 14.6%. Nearby higher transmission study communities (between 2 and 15km away) maintained similar infection profiles to pre-LLIN with mf prevalence ranging from 3.8% in the children <10 years to 21.9% in the older age groups and ICT card positivity of 53.7%. Furthermore, 29% of MF-negative individuals in the study were antibody positive, including 46 of 529 (8.7%) MF-negative children under 10 years of age (considered the sentinel population for exposure). These results indicate that adults remain a reservoir for MF in these communities and antibody assays indicate continued LF transmission, even after the distribution of LLIN. These results indicate that LLIN are insufficient to interrupt transmission in this area and MDA will be necessary to achieve elimination endpoints.

7

IMPACT OF ANNUAL AND SEMIANNUAL MASS DRUG ADMINISTRATION IN AREAS CO-ENDEMIC FOR *BRUGIA TIMORI* AND *WUCHERERIA BANCROFTI* IN EAST NUSA TENGGARA, INDONESIA

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Lymphatic filariasis is targeted for global elimination by the year 2020. Key strategy is the annual mass drug administration (MDA) with single dose of DEC combined with albendazole for at least five years. In order to shorten the duration of MDA, more rounds may be needed to achieve similar outcome. Here we compared the impact of annual and semiannual MDA on the prevalence of *Brugia timori* and *Wuchereria bancrofti* in Sikka District in Flores Island. Two villages (Paga, *B. timori* only and Lewomada, co-endemic) were assigned annual MDA with single dose of DEC/albendazole, while the third village (Pruda, co-endemic) was assigned semiannual MDA. Blood samples were collected from individuals aged 5 years and older before and 1, 2, and 3 years after the first treatment. The overall compliance with MDA was ranging from 67-90%. Microfilaremia (mf) was determined by microscopic examination of thick night blood smears for all years. Detection of filarial-specific IgG4 (Brugia Rapid, BR, for B. timori) or filarial antigen (ICT cart test for W. bancrofti) were performed at baseline and after three years post initial treatment. The mf prevalence in Pruda village decreased after 5 semiannual treatments from 14.2% to 1.3%, while in other two villages the mf prevalence decreased after 3 annual treatments from 3.9 and 5% to 0 and 0.3%, respectively. The ICT positivity rate in Pruda and Lewomada decreased from 22.9 and 6.5% to 7 and 0.8%, while the BR positivity rate in Pruda, Lewomada and Paga decreased even stronger from 28.9, 31.7 and 12.5% to 3.6, 4.1 and 1.8%. Mf prevalence and BR as well as ICT positivity rates show similar levels of reduction in both MDA regimens, but Pruda started out at much higher levels. We conclude that in our setting 3 annual rounds of MDA are sufficient to reduce mf rates to less than 1% in the population, but semiannual MDA is helpful for higher prevalence settings to reach the same degree of reduction.

8

EL NIÑO AND THE SHIFTING GEOGRAPHY OF CHOLERA IN AFRICA

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In the wake of the 2015-2016 El Niño event, there has been an explosion of cholera cases in East Africa, including the largest outbreak since the last strong El Niño (1997-1998) in Tanzania. El Niño conditions are associated with increased rainfall in East Africa and decreased rainfall in Southern Africa, West Africa, and parts of the Sahel. Because of the key role of water supplies in cholera transmission, a relationship between El Niño events and cholera incidence is highly plausible, and previous research has shown a link between El Niño patterns and cholera in Bangladesh. However, there is little systematic evidence for this link in Africa. Using high-resolution mapping techniques, we find that El Niño profoundly changed the annual geographic distribution of cholera in Africa from 2000-2014, shifting the burden to continental East Africa, where almost 50,000 additional cases occur during El Niño years. Cholera incidence during El Niño years was higher in regions of East Africa with increased rainfall, but incidence was also higher in some areas with decreased rainfall suggesting a complex relationship between rainfall and cholera incidence. Here we show clear evidence for a shift in the distribution of cholera incidence throughout Africa in El Niño and non-El Niño years, likely mediated by El Niño's impact on local climatic factors. Knowledge of this relationship between cholera and climate patterns coupled with El Niño forecasting could be used to notify countries in Africa when they are likely to see a major shift in their cholera risk. Effective case management enormously decreases mortality in cholera outbreaks and new control tools (e.g., oral cholera vaccines) may be able to prevent outbreaks altogether. Therefore the ability to step up preparedness and surveillance when local risk is high can have a major impact on saving lives and lowering the disease burden.

9

SURVEILLANCE FOR CHOLERA MORTALITY DURING AN URBAN EPIDEMIC—DAR ES SALAAM, TANZANIA, 2016

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Cholera can cause profuse watery diarrhea, severe dehydration, and death within hours. Effective treatment with oral rehydration salts (ORS) and intravenous fluids (IVF) can lower the case fatality rate from >30% to <1%. In August 2015, a cholera epidemic occurred in Tanzania, and by 27 October 3,773 cases and 36 deaths were reported in Dar es Salaam. Because of rumors of additional deaths in the community, we conducted a mortality investigation. We defined a suspected cholera death as death from acute watery diarrhea in a person ≥2 years old in Dar es Salaam after 15 August 2015. We obtained information about health facility deaths from cholera treatment centers and referral hospitals, and community deaths from municipal burial permits. We interviewed family members about decedents' demographic characteristics, timing and location of death, care-seeking behavior, and treatment. We identified 96 cholera deaths in 2015; 56 (58%) were not captured by surveillance. We were unable to interview family members of 40 (42%) of 96 decedents; 35 (85%) could not be found, 3 (8%) had moved, and one entire family of 2 (5%) died. Family interviews revealed that 56 decedents ranged in age from 2 - 80 years (median 23 years); 32 (57%) were male; 35 (63%) had primary school education or less; and 45 (80%) died within 24 hours of symptom onset. Of 56 decedents, 33 (59%) died in the community or en route to care, 22 (39%) in a health facility, and 1 (2%) in an unknown location. Ten percent of decedents reportedly took ORS at home after becoming ill, and 31% waited >6 hours to seek care. Of 43 decedents who sought care at a health facility, 15 (35%) received neither ORS nor IVF. Of 33 community decedents, 24 (72%) had sought care at a health facility and were discharged before death. Most cholera deaths were not captured by surveillance. ORS use at home was inadequate, care-seeking was often delayed, and most community decedents were discharged from a health facility before death. For many decedents treated in health facilities, rehydration therapy was inadequate. To address these problems, case management trainings were held and cholera messages disseminated to the population.

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THE PLASMA AND MUCOSAL ANTIBODY RESPONSE TO THE COMPLETE *VIBRIO CHOLERAE* O1 PROTEIN IMMUNOME AND O-SPECIFIC POLYSACCHARIDE IN ADULTS WITH INABA OR OGAWA CHOLERA IN BANGLADESH

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Cholera is a severe dehydrating illness of humans caused by *Vibrio cholerae* O1 serotype Inaba or Ogawa. Characterization of immunogenic *V. cholerae* antigens could lead to a better understanding of protective immunity in human cholera infection. Using microarrays, we immuno-probed > 3500 *V. cholerae* antigens with plasma and antibody-in-lymphocyte supernatant (ALS) collected from 7 adult Bangladeshi cholera patients infected with *V. cholerae* O1 (4 Ogawa; 3 Inaba). Antibodies in ALS peak within a week of infection, and reflect transiently circulating activated lymphocytes migrating to lamina propria; as such, antibodies in day 7 ALS are surrogate markers for humoral responses in intestinal tissue. Overall, we identified 151 *V. cholerae* O1 immunoreactive antigens (61 in plasma and 103 in ALS). We rank ordered immunoreactivity, and identified 16 antigens with significantly higher immunoreactivity at convalescence (day 7 and day 30) compared to acute phase plasma (day 2), and 18 antigens with higher immunoreactivity in ALS of cholera patients collected at day 7 compared to age, gender, and ABO-matched healthy Bangladeshi controls. A number of the identified antigens have previously been demonstrated to be immunogenic and associated with virulence (e.g. *V. cholerae* O-specific polysaccharide (OSP); cholera toxin A and B subunits (CtxA, CtxB); toxin co-regulated pilus A (TcpA); and *V. cholerae* cytolysin, VCC/HlyA). Additional identified antigens included sialidase (NanH), a virulence factor required for mucosal colonization; and flagellin proteins FlaC and FlaD, involved in motility. Thus far we have confirmed plasma immunoreactivity to sialidase and FlaC via standard ELISA, and are in the process of confirming immunoreactivity of these and other identified antigens using ALS. This study is the first profiling of the mucosal and systemic antibody responses to the complete *V. cholerae* O1 protein immunome and O-specific polysaccharide. Our analysis has identified novel antigens that may be involved in host-pathogen interactions, and the results may aid in the development of an improved cholera vaccine.

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THE EARLY B CELL RESPONSE TO THE *VIBRIO CHOLERAE* O1 ANTIGEN IS CHARACTERIZED BY A HIGH DEGREE OF CLONALITY, SOMATIC HYPERMUTATION AND RECALL OF PRIOR ANTIGEN EXPOSURE

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Vibrio cholerae is non-invasive, induces long lasting protective immunity, and is an example of a highly effective human mucosal immune response. Plasmablasts, or early activated antibody secreting cells, which are found briefly in the circulation after cholera are strongly predictive of subsequent duodenal responses, suggesting that these cells provide a transient window into immunologic memory at the mucosal surface. In this study, we investigated early B cell responses to cholera using a single-cell, single-antibody analysis of the immunoglobulin repertoire, specificity, and functional characteristics of cholera induced plasmablasts. Analyzing an array B-cell receptor sequences and a panel of 140 monoclonal antibodies generated from these sequences, we found that cholera induces a plasmablast response marked by high levels of somatic hypermutation and clonality, and that the majority of B cell expansions following cholera produce antibodies that target the immunodominant antigens of *V. cholerae*: CT and LPS. In addition, *V. cholerae* sialidase was a novel major target of the early B cell response following cholera. We found that effective cholera toxin neutralizing responses targeted both the A and B subunits, and were likely impacted by prior exposure to Enterotoxigenic *E. coli* in the study population. Most notably, *V. cholerae* O1 LPS responses uniformly target the O-specific polysaccharide, but varied widely in serotype specificity and functional characteristics. Unexpectedly, we found that antibodies which bind preferentially to the previously circulating *V. cholerae* O1 Inaba serotype were characterized by high levels of somatic hypermutation and but additional mutations provided the ability to adapt to the Ogawa serotype. These findings suggest the existence of bona fide immunologic memory against a canonical T-cell independent antigen and provide an underlying mechanism for the long term immunity seen following cholera.

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IMMUNE RESPONSE TO ORAL CHOLERA VACCINE (SHANCHOL) IN INTERNALLY DISPLACED PERSONS IN SOUTH SUDAN

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Large scale outbreaks of cholera outside of historically endemic regions have renewed interest in new cholera control toolkits, including oral cholera vaccines (OCV). Extensive evidence supports the use of OCV in South Asia where the disease is endemic, but there is a dearth of efficacy studies outside of historically endemic regions where differential demographics, health status and co-circulation of other pathogens could influence vaccine response. We conducted an immunogenicity study during a 2015 pre-emptive OCV campaign in internally displaced persons (IDPs) in South Sudan. We report the immunological responses to Shanchol in a subset of volunteers (n=205), the impact of age, one versus two OCV doses and baseline titers on vaccine response. Consistent with recent circulation of cholera, high baseline titers (>80) were observed in 21% of the study participants. Amongst those without evidence of recent exposure to cholera (baseline vibriocidal titers ≤80), 90% of young children, 73% of older children and 72% of adults seroconverted (≥4 fold changes in vibriocidal titers) after the 1st OCV dose against serotype Inaba; with similar percentages of individuals seroconverting after 2nd dose; responses against serotype Ogawa were similar. Immunological endpoints (vibriocidal titers, isotype antibody levels) did not differ between the 3 age

groups post vaccination suggesting the beneficial effects of Shanchol in all. Adults and older children had baseline titers >80 more frequently than younger children (consistent with higher probability of previous cholera exposure), which was inversely associated with seroconversion. For all age groups, responses did not differ significantly between one or two doses of vaccine. Immunological priming (high baseline titer) is reflective of the increasing endemicity of *Vibrio cholerae* in South Sudan. Our results indicate Shanchol is immunogenic in a cohort of internally displaced individuals in South Sudan, and that a single dose alone may be sufficient to achieve a similar immunological response as the currently licensed two-dose regimen.

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THE EFFECTIVENESS OF ONE DOSE OF ORAL CHOLERA VACCINE IN RESPONSE TO AN OUTBREAK

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Oral cholera vaccines (OCV) represent a new effective tool to fight cholera and are licensed as two-dose regimens with 2-4 weeks between doses. Evidence from immunologic studies and secondary analyses from epidemiologic studies suggests that a single-dose of OCV may provide significant protection against cholera. A one-dose regimen, if effective, would be cheaper and easier to use in outbreaks to rapidly protect large at-risk populations. During a cholera outbreak starting in May 2015 in Juba, South Sudan, the Ministry of Health, Médecins Sans Frontières and partners engaged in the first field deployment of a single-dose of OCV (Shanchol®) to enhance their outbreak response. We conducted a case-cohort study to estimate the short-term effectiveness of one-dose of Shanchol, enrolling suspected cholera cases from 9-August-2015 through 29-September-2015 and an 898-person cohort. Suspected cholera was confirmed through multiple diagnostic tests including PCR and culture. Unadjusted and adjusted vaccine effectiveness were estimated with proportional-hazards regression models. We enrolled 87 suspected cases into the study from cholera treatment centers throughout Juba with 34 classified as cholera positive, 52 as cholera negative and 1 with indeterminate results. None of the 858 cohort members who completed a follow-up visit developed clinical cholera during follow-up. The unadjusted single-dose effectiveness was 80·2% (95% CI 61·5,100·0) and after adjusting for potential confounders, 87·3% (95% CI 70·2,100·0). One dose of Shanchol was effective in preventing medically-attended cholera in this study. These results support the use of single-dose strategy in response to outbreaks.

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ORAL CHOLERA VACCINE STUDIES IN HIGH CHOLERA ENDEMIC SETTINGS IN BANGLADESH

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Bangladesh bears the highest burden of endemic cholera with an estimated 450,000 cholera cases and over 4,500 deaths annually. From 2011 to 2016 large scale vaccination studies have being conducted with the oral cholera vaccine (OCV) ShancholTM in high risk urban areas in Bangladesh. The objective these studies is to assess the feasibility of delivery and vaccination strategies utilizing the existing national immunization facilities. To study effectiveness, thermal stability, optimal dosage, host factors including immune responses and interval of vaccination needed for making the OCV program successful in Bangladesh. Vaccination with a 2 dose of Shanchol through the routine public health care system in urban settings of Bangladesh is feasible, acceptable and impactful with over 50% protection evident in all age groups and sustained for 2 years. In the rural settings the program was successful and 92% of the 1st dose recipients received 2nd dose of OCV. A Phase III protective efficacy study carried out in urban slums of Dhaka city among 205,600 participants and efficacy measured one year and above. Overall results from these studies have shown that OCV is safe and satisfactory immune response is elicited after intake of vaccine stored in the cold or at elevated temperatures of storage. This latter observation makes the cold chain not obligatory for vaccine delivery in large campaigns such as in epidemics and outbreaks. The vaccine could be delivered to people in high risk densely populated settings in over 500,000 people in children and adults with support of the EPI. However, to better understand the optimal strategy for vaccination the population to immunize against cholera; surveillance for cholera is ongoing in 22 sites in Bangladesh to gauge the prevalence of the disease. In summary to implement OCV uptake in national immunization programs factors such as the target age group to vaccinate, feasibility of program, cost-effectiveness as well as the availability of enough cholera vaccine doses as funding to meet the large demand for a successful and sustainable OCV program in the country.

15

EVIDENCE TO OPTIMIZE THE DESIGN OF SCHOOL-BASED INTERVENTIONS AGAINST MALARIA

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In Malawi, the prevalence of *Plasmodium* infection is highest in school-age children, making this age group an attractive target for interventions to decrease the burden of malaria. However, prior trials of school-based malaria control interventions have had mixed results. Two potential reasons for intervention failures are insensitive diagnostics used in screen-and-treat efforts, and/or frequent reinfection following the intervention. We conducted school-based cohort studies to further evaluate the dynamics of *Plasmodium* infection in this age group and to help improve intervention efficacy. Students were randomly selected and enrolled in observational, school-based, cohort studies in the rainy (N=405) and dry (N=381) seasons of 2015 in Malawi. All students were followed at 1, 2, and 6 weeks after baseline, using surveys of malaria symptoms and bed net use, microscopic examination of blood smears, and PCR testing of dried blood spots. At baseline, students with positive rapid diagnostic tests (RDTs) were treated with artemether-lumefantrine. Thirty-eight percent of participants were RDT-positive and treated at baseline and an additional 11% had PCR-

based infection that was below the limit of detection (LOD) of RDTs (Negative predictive value 81%, CI: 77-85%). Among students who were RDT positive and treated at baseline during the rainy season, 35% were RDT-positive, and 44% were PCR-positive at week 6. Among RDT-negative, PCR-positive students who were not treated at baseline, 75% had infection detected at least one more time during the 6-week follow up. Nearly one quarter were PCR-positive at each of the four visits. More detailed longitudinal analysis and dry season results will also be presented. RDTs frequently do not detect low-density *Plasmodium* infections. However, the high rate of reinfection following treatment in school-age children highlights the need to tailor the timing of treatment intervals to the prophylactic period of the drug and the epidemiologic context.

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EVALUATION OF A PRIMARY SCHOOL-BASED MALARIA CASE MANAGEMENT PROGRAM ON SCHOOL ATTENDANCE IN SOUTHERN MALAWI

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Despite school-age children harbouring a large proportion of malaria parasites in the community, evidence suggests they are the age-group least likely to be taken for treatment at a health facility, or to sleep under a bed net. Evidence also indicates malaria is an important cause of morbidity in school children and a potentially significant contributor to school absenteeism. Primary schools present a pragmatic opportunity to address this disease burden and improve access to malaria diagnosis and treatment in this age group. A cluster randomized controlled trial was conducted in 58 primary schools in Zomba District. In 29 randomly selected schools, 2-4 teachers were selected to diagnose and treat uncomplicated malaria using rapid diagnostic tests (RDTs) and artemisinin-based combination therapy (ACT) as part of a basic first aid kit (Learner Treatment Kit or LTK). School attendance of children in Standards 2, 4 and 6 in was monitored using daily teacher-recorded registers and unannounced attendance 'spotchecks' by the study team. Prevalence of malaria parasitaemia, anaemia and educational performance was assessed at the end of the study. Between December 2013 to March 2015, 92 trained teachers in 29 primary schools provided 32,193 unique consultations to school children seeking care. During the peak transmission season significantly more children sought care; fulfilled diagnostic criteria for testing by RDT; and were found malaria positive. Despite a significantly greater proportion of consultations provided to female children between 6 and 14 years old, no difference was observed in the type of symptom reported. No significant difference was observed in the proportion of child-days recorded as absent in teacher registers (unadjusted OR=0.90 (0.59-1.36), p=0.614) or of children absent during 'spotchecks' throughout the intervention. There was no significant difference in prevalence of malaria parasitaemia, anaemia or education scores between the groups at the end of the intervention. In spite of this apparent lack of impact this programme of school-based malaria case management was a highly utilised and acceptable source of care.

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FACILITY-BASED ENHANCED MALARIA SURVEILLANCE TO MEASURE VECTOR-CONTROL INTERVENTION IMPACT IN WESTERN KENYA, 2012-2015

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Reliable monitoring and evaluation platforms to measure the impact of malaria control interventions are important for programs. We implemented enhanced malaria surveillance at 10 health facilities in western Kenya to monitor the impact of discontinuing indoor residual spraying (IRS). One health facility in 10 sub-counties was purposefully selected for enhanced surveillance for 1 week per month from August 2012 to April 2015. We collected clinical data and malaria rapid diagnostic test (RDT) results for all persons in outpatient clinics with a documented temperature of ≥37.5°C or history of fever. We compared the change over time in facility-specific malaria test positivity rates in relation to historical IRS implementation. During 155 enhanced-surveillance days over 33 months, 84,365 persons presented to facilities; 65.1% fit criteria for suspect malaria, of whom 45.1% were RDT positive. Older children ages 5-14 years (66.4%) were more likely to have confirmed malaria than children ages <5 years (49.1%; OR=1.35; 95% CI: 1.25-1.45; p<0.0001). Males of all ages (48.4%) were more likely to have confirmed malaria than females (42.6%; OR=1.14; 95% CI: 1.11-1.17, p-value <0.0001), which was most pronounced in males aged ≥15 years. The test positivity rate was significantly lower (31.5%) in IRS sub-counties in the first 9-months after spraying compared to the following 9 months (42.7%) after discontinuing IRS (OR=0.74, CI: 0.62-0.88, p=0.0008). Over the surveillance period, there was no difference in malaria positivity rate trends between facilities in historical IRS areas (i.e., with 1-5 years of spraying) and those without IRS. Monitoring the effects of a dynamic vector-control strategy was facilitated by enhanced malaria surveillance at a few facilities. Males and older children were most likely to have confirmed malaria perhaps because of prevention efforts focused on pregnant women and young children. Although reductions in malaria positivity rates were not detected as a long-term effect of IRS, the results highlight the importance of complementary vector-control activities as priorities and strategies shift.

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COMPARISON OF THREE VERSUS FOUR ROUNDS OF SEASONAL MALARIA CHEMOPREVENTION ON THE INCIDENCE OF CLINICAL MALARIA IN MALI

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Seasonal malaria chemoprevention (SMC) is a simple and effective strategy recently recommended by WHO for malaria control in children between 3 to 59 months living in Sahelian countries with seasonal transmission

like Mali. SMC entails administration of curative doses of sulfadoxine -pyrimathamine and Amodiquine at monthly intervals during the high transmission season. SMC efficacy in Mali, Senegal and Burkina Faso was demonstrated with three treatment courses, and there are no data to support additional benefit provided by a 4th treatment course ass suggested in the WHO recommendation. Considering the logistics of an additional treatment course, we sought to determine the benefit of 4 versus 3 courses of treatment. Children aged 3-59 months in 17 villages in two health sub-districts in Oulelessebougou were randomized to receive either 3 or 4 SMC rounds during the transmission season, starting in August 2015 using the door-to-door delivery method. Incidence rate of clinical malaria over the transmission season (August to December) measured by passive surveillance was compared between the arms. Overall, 3578 children were enrolled and followed during the 2015 transmission season. The incidence rate of clinical malaria was 0.26 episodes/child/season in children who received 3 courses of SMC and 0.20 episodes/child/season in those who received the 4th SMC treatment course, corresponding to a reduction of 25% in incidence of clinial malaria in chidren who received the 4th SMC treatment course (incidence rate ratio (IRR) = 0.75 , 95% Confidence Intervals (CI) 0.63-0.90, p = 0.002). After adjustment for age and gender, using negative binomial regression, the reduction remained unchanged (IRR = 0.75, 95% CI 0.63, p = 0.001). A fourth treatment course of SMC during the malaria transmission season provided additional protection against malaria clinical episodes.

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SUBPATENT MALARIA INFECTION IS NOT ASSOCIATED WITH POOR BIRTH OUTCOMES IN KENYAN WOMEN RECEIVING INTERMITTENT SCREENING AND TREATMENT OR INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN PREGNANCY

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Malaria in pregnancy has significant adverse consequences. However, the impact of subpatent infections on pregnancy outcomes remains unclear. We assessed the impact of subpatent infections in a longitudinal cohort of women enrolled in a study evaluating the efficacy of intermittent screening and treatment in pregnancy with dihydroartemisinin-piperaquine (ISTp-DP), and intermittent preventive treatment in pregnancy (IPTp) with DP compared to IPTp-sulfadoxine-pyrimethamine (SP). HIV-negative women were enrolled at 12-32 weeks gestation. At each visit, samples were collected for malaria detection by microscopy and polymerase chain reaction (PCR), and by RDT for women in the ISTp-DP arm. Logistic and linear regression were used to assess the cumulative effect of patent and subpatent infections (compared to no infection) on birthweight (BW), low birth weight (LBW, <2500 gm), maternal haemoglobin (Hb) at delivery, and maternal anemia (Hb<11 g/dL), adjusted for relevant covariates. Among 1523 singleton pregnancies, 54% were paucigravid women (G1/2) and 46% multigravid women (G3+); 33% had malaria by PCR at enrolment. Neither patent nor subpatent parasitemia was associated with LBW (adjusted odds ratio (aOR) and 95% CI for patent: 1.2, 0.45-3.33; subpatent: 0.3, 0.03-1.87, or mean BW (adjusted mean difference (MD) and 95% CI for patent: -101g, -213 to 11; subpatent: 86g, -21- 192). Both patent and subpatent parasitemia were associated with reduced mean maternal Hb, though this was significant only for patent (MD, 95% CI for patent: -0.50g/dL, -0.87- -0.13; subpatent: -0.15, -0.50, 0.20). Subpatent parasitemia was associated with an increased risk of maternal anemia among G1/2 (aOR 2.9, 1.5-5.8) but not G3+ (aOR 0.75, 0.34-1.66); patent parasitemia was not significantly associated with anemia in either G1/2 or G3+ (aOR 1.61, 0.83-3.12 and 1.48, 0.62-3.57, respectively). In the context of routine screening (ISTp-DP) or IPTp in a high malaria transmission setting in western Kenya, we found no evidence that patent or subpatent parasitemia were associated with LBW or mean birthweight, despite associations with maternal anemia and Hb.

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IGNORING PEOPLE 'WHO ARE NOT THERE' MAY MITIGATE SUCCESS OF MASS DRUG ADMINISTRATION FOR MALARIA: FINDINGS FROM A MIXED-METHOD STUDY IN THE GAMBIA

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The Gambia has achieved great reductions in malaria morbidity but complete elimination still presents a challenge. Mass drug administration (MDA) has been proposed for eradication of malaria in low- and moderate-transmission settings but no MDA programme has been successful in sub-Saharan Africa. This social science study aimed to determine the consequences of the assumption that rural villages are static entities and exclusion of mobile people from MDA on malaria transmission in villages. The study was nested into a cohort study in twelve villages in The Gambia, which implemented two MDA campaigns. In sequential mixed-methods study design a quantitative survey in four villages complemented findings from qualitative research. Active case-finding identified individuals not enrolled in the cohort but present in the village at the time of study. 1384 people from four villages were enrolled in the cohort at baseline. In December 2015, 112 individuals who stayed in the villages but were not included in the MDA cohort were interviewed and screened for parasiteamia. The main reasons for not being included in the cohort study were mobility (travelled or moved); not meeting the study's eligibility criteria; withdrawal or lack of awareness. Among surveyed individuals 9.8% were parasitemic and 74.1% were not adequately protected by bed net. Males (OR=1.14, P<0.05) had increased odds of malaria parasitemia and sleeping under long-lasting insecticide-treated net (LLIN) was protective from malaria (OR=0.7, P=0.007). The findings confirm the importance of human mobility for malaria elimination in two ways. Mobile people might constitute a reservoir of malaria infection that is missed but in addition the people not involved in the cohort study used little protective measures and were thus at increased risk of infection. The use of census lists to identify the beneficiaries could mitigate success of MDA interventions. To achieve sustainable reduction of malaria, MDA interventions have to take into consideration the fluidity of villages and expand their eligibility criteria to include targeting people who are mobile and may not be documented as residents.

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COST-EFFECTIVENESS OF FOCAL MASS DRUG ADMINISTRATION AND MASS DRUG ADMINISTRATION WITH DIHYDROARTEMISININ-PIPERAQUINE FOR MALARIA PREVENTION IN SOUTHERN PROVINCE, ZAMBIA: RESULTS OF A COMMUNITY RANDOMIZED CONTROL TRIAL

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The Zambian National Malaria Elimination Program has embarked on a national strategy of malaria elimination because of recent successes and challenges in sustaining malaria control efforts. As part of this strategy, community-based treatment approaches, combined with vector control, are being tested in a large scale trial with the intent of possibly interrupting malaria transmission and reducing the burden of malaria in southern Zambia. The trial compared three arms: 1) Standard of Care including high vector control coverage with LLIN and IRS using Actellic®, roll-out of community case management for malaria and efforts to improve the quality of diagnostics and treatment, 2) Mass Drug Administration (MDA) using dihydroartemisinin-piperaqunie (DHAp) and 3) Focal Mass Drug Administration (fMDA) with DHAp. Cost was measured at the health facility catchment level to estimate the costs and cost-effectiveness of the MDA and fMDA strategies. Results differed by outcome (infections averted vs. clinical cases averted) and transmission strata (high vs. low), but in all cases MDA showed superior cost-effectiveness to fMDA. Cost-effectiveness acceptability curves produced in probabilistic sensitivity analysis indicated that both MDA and fMDA would be highly likely (>80%) to be considered cost-effective interventions (≤3x GDP per capita per disability adjusted life year (DALY) averted by WHO standards when infections averted were used as a basis for modelling DALY outcomes, but that neither intervention was considered highly cost-effective in these settings (<1x GDP per capita per DALY averted).

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DISCOVERY AND DEVELOPMENT OF A MULTISTAGE ANTIMALARIAL WITH NEW MECHANISM OF ACTION USING NEXT GENERATION SYNTHESIS

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Despite increased efforts in the last decade, the antimalarial drug discovery and development pipeline still lacks compounds with non-erythrocyte stage activity or with novel mechanism of action (nMOA). Antimalarial leads have thus far been derived mainly from two sources - natural products and synthetic 'drug-like' compounds. At the Broad Institute, we created a diverse collection of synthetic compounds having three-dimensional features reminiscent of natural products and underrepresented in typical screening collections (Diversity Oriented Synthesis compound collection). A novel series, the bicyclic azetidine, was found with robust *in vivo* efficacy in erythrocytic, hepatic and sexual stages. The compound series inhibits phenylalanine t-RNA synthetase activity, a novel molecular target, with a low propensity to induce resistance and shows efficacy with a single dose efficacy in both *Plasmodium berghei* and NSG *P. falciparum* models. Extensive pharmacokinetic and preclinical safety data supports the progression of this novel antimalarial agent towards pre-IND development.

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TARGETING RESISTANCE: EXPLOITING EVOLUTION IN DRUG DISCOVERY

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Significant strides have been made in the past decade to control malaria, however these fragile gains are in danger of being lost due to the rise and spread of ACT resistance. New strategies are needed to combat resistance and extend the lifespan of antimalarial chemotherapies. As evidenced by the emergence of ACT failure, traditional approaches of ad hoc drug combinations are not sufficient. Here we explore a new paradigm for protecting the efficacy of antimalarial therapies by identifying partner drugs that decrease the selective advantage of drug resistant cells from emerging. We propose suppressing resistance with a population biology trap: by identifying situations where resistance to one compound confers hypersensitivity to another, we can design combination therapies that not only kill the parasite, but also guide its evolution away from resistance. We applied this concept, termed "targeting resistance," to the malaria enzyme dihydroorotate dehydrogenase (PfDHODH). We have demonstrated that resistance mutations quickly arise from *in vitro* parasite selection with single PfDHODH inhibitors. Characterization of these resistant parasites showed that resistance to one PfDHODH inhibitor rendered them hypersensitive to other structural classes. To further develop this concept, we performed a high-throughput screen to identify inhibitors selective for mutant PfDHODH. As part of a Tres Cantos Open Lab Foundation project, we screened select GSK libraries and identified 25 mutant-selective and 74 wild-type-selective compounds. Of particular interest are an additional 28 compounds that were equally potent against both the wild-type and mutant enzymes. These molecules were then validated in cellular assays and prioritized candidates identified, representing promising starting points for further development. Furthermore, pairing wild-type and mutant-selective PfDHODH inhibitors largely suppressed the emergence of resistant parasites in *in vitro* experiments. We believe that this approach is widely applicable to other antimalarial targets and represents a new drug development strategy for a variety of diseases.

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A NEW BENZOXABOROLE WITH AN APPARENT NOVEL MECHANISM OF ACTION AGAINST *PLASMODIUM FALCIPARUM*

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New antimalarial drugs are needed. We have synthesized several oxaboroles with nanomolar activity against cultured malaria parasites and excellent efficacy in murine malaria models. AN13762 is a current lead candidate. It was active against multiple laboratory strains (IC50 for Dd2: 50 nM, W2: 30 nM, 3D7: 41nM, 7G8: 70 nM, and VS1: 73 nM) and fresh field isolates of *Plasmodium falciparum* (mean IC50 91nM). Pharmacokinetic assessment showed that AN13762 was ~100% orally bioavailable with t1/2 5-10 h in mouse, rat, and dog. AN13762 had

potent activity in murine models, with ED90 6.3 mg/kg against *P. berghei* and 0.85 mg/kg against *P. falciparum*-infected mice, with *in vivo* rates of clearance similar to those for artesunate. No genetic toxicology safety concerns for AN13762 were identified in Ames assays or *in vivo* rat micronucleus studies. Cytotoxicity was not seen at concentrations up to 100 μM in human cell lines. Treatment of cultured *P. falciparum* with AN13762 for 8 h intervals across the erythrocytic life cycle demonstrated maximal activity against rings and trophozoites, and parasites did not develop beyond this stage. To gain further insight into mechanisms of action we selected *P. falciparum* with decreased sensitivity to AN13762 by culturing Dd2-strain parasites in step-wise increasing concentrations or a single high concentration of AN13762. Parasites selected rapidly for resistance, with IC50 values of 400-2500 nM after selection. AN13762-resistant parasites were analyzed by whole genome sequencing. Compared with sensitive parental parasites, resistant parasites consistently had SNPs in genes predicted to encode a microtubule and actin binding protein (PFC0960c), lysophospholipase (PF07_0040), SUMO-activating enzyme subunit 2 (PFL1790w), and a protein of unknown function (PF14_0594). In summary, the benzoxaborole AN13762 represents a promising new class of antimalarial compounds, for which the mechanism of resistance appears to be complex.

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NOVEL CLINICAL STUDY AND PHARMACOMETRIC MODELLING TO FIND THE MINIMUM INHIBITORY CONCENTRATION (MIC) OF A NEW ANTIMALARIAL

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Determination of the MIC of antimalarial drugs in the clinical setting may provide a better alternative to empirical approaches to effective dose finding. This was an open-label, dose-ranging (de-escalation), phase IIa study in Vietnamese adults (n=25) with uncomplicated *Plasmodium falciparum* malaria to estimate prospectively the in-vivo MIC of cipargamin. Patients were treated sequentially with single cipargamin doses (30, 20, 10, or 15 mg). Population PK/PD modelling of plasma cipargamin concentrations, serial parasite densities assessed by microscopy and real-time quantitative PCR were used for estimating the in-vivo MIC. PK properties of cipargamin were described by a flexible transit-absorption model followed by a one-compartment disposition model, resulting in a high predictive performance. Individual PK estimates were then imputed into the PK/PD model to assess the cipargamin-dependent parasite killing effect. As no parasite growth data were available before drug administration, the parasite multiplication rates were fixed to 10-fold multiplication/parasite life-cycle (48 hours) based on malariatherapy and volunteer data. Initial implementation of the PK/PD model assumed a homogenous parasite population and drug-dependent killing of parasites (EMAX model). Population and individual parasite clearance curves showed a biphasic pattern of parasitaemia decline, suggesting the presence of dormant (non-sensitive) parasite population. The fraction of sensitive/dormant parasites and the activation of dormant parasites were estimated. Higher doses and plasma cipargamin concentrations were associated with significantly faster maximum parasite killing. A total of 23 patients were characterized accurately as either cured, having had early treatment failure or recrudescent infection using the final model. Median (range) MIC was estimated at 0.126 (0.0375-0.803) ng/mL occurring at median (range) time of 7.45 (5.29-11.4) days. The developed PK/PD model demonstrated an informative approach for determining the in-vivo MIC, providing a rational framework for dose finding in antimalarial drug development.

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ASSESSING THE SPEED OF CLEARANCE OF *PLASMODIUM VIVAX* FROM THE BLOOD FOLLOWING TREATMENT WITH A LICENSED AND EXPERIMENTAL ANTIMALARIALS

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The rate of clearance of malaria parasites from the blood of patients is a key determinant of treatment success. For example, in falciparum malaria the rate of clearance in early infection is inversely associated with mortality. Although it is considered that drug response in *Plasmodium vivax* infection is generally faster, there is a paucity of information, both because *in vitro* culture is not possible and because clinical trials have been fewer. To investigate the relative activity of four antimalarials against *P. vivax* we undertook clinical trials entailing induced blood stage infection with *P. vivax*. Artemether/lumefantrine (A/L) and chloroquine (CQ) were administered at the approved doses, while artefenomel (OZ439) and DSM265 are scheduled to be administered as a single dose of 200mg and 400mg, respectively. Subjects were treated at symptom onset, and rate of clearance of asexual parasitemia measured by qPCR. Parasite clearance half life (PCt1/2) and parasite reduction ratio (PRR) were derived from the slope of the parasite clearance curve. Data from 10 subjects treated with A/L indicate a PCt1/2 of 1.6 hrs (95%CI: 1.5-1.7) and a Log10PRR of 9.1 (95%CI: 8.4-9.7). Data from 8 subjects treated with CQ indicate a PCt1/2 of 5.0 hrs (95%CI: 4.7-5.4) and a Log10PRR of 2.9 (95%CI: 2.7-3.1). Similar data from the OZ439 and DSM265 cohorts will be available for presentation. Deriving these key pharmacodynamics variables of antimalarials against *P. vivax* will be of major assistance in selecting optimal drugs, combinations, doses and regimens so that optimal systems are developed for treatment of this widely prevalent and important pathogen.

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FOSMIDOMYCIN-PIPERAQUINE AS NON-ARTEMISININ-BASED COMBINATION THERAPY FOR ACUTE UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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The combination of fosmidomycin and piperaquine, with the attributes of rapid blood schizonticidal activity and prolonged post-treatment prophylaxis, is being developed to meet the challenge of emerging artemisinin resistance. As a potent inhibitor of 1-Deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase, fosmidomycin possesses a unique mode of action through blockade of the non-mevalonate pathway of isoprenoid biosynthesis. In contrast, piperaquine is thought to bind to heme, inhibiting its detoxification within the malaria parasite. It is further postulated that the toxic build-up of heme increases the membrane permeability of red cells favouring the influx of fosmidomycin. The efficacy, tolerance and safety of the combination for the treatment of acute uncomplicated *Plasmodium falciparum* mono-infection are being evaluated in a proof of concept study in Gabon. A total of 100 symptomatic patients including 10 adults aged >14 years, 40 older children aged 5 to 14 years, and 50 younger children aged one to five years with initial parasite counts between 1,000 and 150,000/μL have been treated with fosmidomycin, in twice daily doses of 30mg/kg, and piperaquine, in a once daily dose of 16mg/kg, orally for three days and followed-up for 63 days. The primary efficacy endpoint is the per protocol PCR-corrected Day 28 cure rate.

Preliminary results at all ages show the combination is highly effective with an excellent tolerance and there are no safety concerns. The full results will be presented at the ASTMH meeting in November 2016. Meanwhile, proposals are being drawn up for dose optimisation studies aimed at achieving a therapeutic regimen of once daily dosing administered over three days.

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MALARIA, MALNUTRITION, AND ADVERSE BIRTH OUTCOMES AMONG PREGNANT WOMEN: A POOLED ANALYSIS

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Malnutrition and malaria infection commonly co-exist, afflicting pregnant women in resource-poor settings. Prior small studies have indicated that the effect of malaria on low birthweight (LBW; <2500g) may depend upon maternal nutritional status. We investigated the interaction between malaria infection during pregnancy and maternal nutrition with regards to the risk of LBW using data from 14,635 singleton, live birth pregnancies from women who participated in 13 pregnancy studies conducted in malaria endemic countries across Africa and Asia from 1996-2015. Study-specific effect estimates and measures of interaction were calculated using linear and log-binomial regression models, adjusted for confounders (maternal age, gravidity, area of residence, HIV infection, anemia) using inverse probability of treatment weights, and pooled across studies using a restricted maximum likelihood random effect model. Nine of the thirteen studies assessed malaria (microscopy or RDT) and mid-upper-arm circumference (MUAC) at enrollment. Across these 9 studies, 75% of women were well-nourished (MUAC≥23 cm) and malaria-uninfected at enrollment, 10% were well-nourished but malaria infected, 12% were malnourished and not malaria infected, and 2% were both malnourished and malaria infected. Compared to women who were well-nourished and uninfected, the pooled risk ratios for LBW were: malaria alone, 1.18 (95% confidence interval [CI]: 0.93, 1.48); malnutrition alone, 1.55 (95% CI: 1.29, 1.85); and malaria and malnutrition together, 1.75 (95% CI: 0.90, 3.37). The pooled interaction contrast was -0.03 (95% CI: -0.11, 0.06; p=0.57), with minimal statistical heterogeneity across studies (=0.0014, Cochran Q=11.63 [p=0.11]). While MUAC<23cm was associated with an increased risk of LBW, malaria infection at enrollment was not as strongly associated and there does not appear to be synergism between these two factors. Additional analyses to be presented will consider: mean birth weight; preterm birth; malaria infection at delivery; malnutrition defined by BMI; meta-regression for subgroup effects; selection bias by excluding pregnancy loss.

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ASSESSING ULTRASONOGRAPHY AS A DIAGNOSTIC TOOL FOR PORCINE CYSTICERCOSIS

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Neurocysticercosis caused by the pork tapeworm, *Taenia solium*, causes 30% of epilepsy in poor rural communities of the developing world. Use of the ring strategy is a promising control intervention targeting treatment of humans and pigs living near heavily infected pigs. Tongue examination for *T. solium* cysts provides a crude means of identifying heavily infected pigs. However, as prevalence decreases over time in treatment communities, higher sensitivity methods are needed to achieve full treatment coverage. This study evaluates ultrasonography as an alternative method to detect pigs infected with varying burdens of *T. solium* cysts. We collected blood samples and purchased 158 seropositive pigs living in eight villages of Piura, a province of northern Peru where *T. solium* is endemic. Tongue examination and ultrasonography of the limbs were performed in these animals, followed by fine dissection necropsy to determine cyst burden. We used necropsy as a gold standard and compared the sensitivity and specificity of ultrasonography with tongue examination for their ability to detect heavy infection (≥ 100 viable cysts) in pigs. Compared to tongue examination, ultrasonography was more sensitive (92% vs. 83%) but less specific (90% vs. 97%) detecting pigs with heavy cyst burdens, although these differences were not statistically significant. The improved sensitivity of ultrasound resulted in the detection of one additional heavily infected pig compared to tongue examination, but also resulted in more false positives (14 vs. 3) due to poor specificity. Ultrasonography was highly sensitive in detecting pigs with heavy cyst burdens and may allow for better treatment coverage in endemic areas compared to tongue examination. In its current form, however, the high rate of false positives results in a substantial number of unnecessary treatments, and must be improved before ultrasound can replace tongue examination as the preferred screening tool for pigs in ring strategy interventions. With improvements in training and technology, the use of ultrasound could potentially benefit local elimination strategies where previous efforts have stalled.

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VASCULAR LEAKAGE IN THE BRAIN IN PORCINE NEUROCYSTICERCOSIS IS ASSOCIATED WITH ANGIOGENESIS

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Anthelmintic therapy for neurocysticercosis, brain infection by *Taenia solium* cysts (larvae), frequently exacerbates neurological symptoms. We have used naturally infected pigs and Evans Blue (EB) to show that the acute post treatment immune response is associated with increased vascular permeability of the blood-brain barrier (BBB). Here we studied potential mechanisms for this increase. We used 11 infected pigs, 3 controls (T0) and 8 treated with a single dose of Praziquantel, euthanized 2 and 5 days later (T2 and T5). EB was injected 2h before sacrifice; biopsies of brain tissue around cysts (capsules) were analyzed using immunohistochemistry (IHC) and real time quantitative PCR for selected markers.EB-stained (blue), "leaky" capsules from all groups had more newly formed vessels (strong IHC reaction for cadherin, vascular endothelial growth factor (VEGF) and von Willebrand factor (vW)) than clear ("non-leaky") capsules and also had higher expression of VEGF, vWf, transforming growth factor beta and ephrine genes, as evidence of angiogenesis. Immature new vessels, with incomplete basal lamina and weak cellular junctions, demonstrated perivascular lymphocytic cuffing, with clear recruitment of immune cells, consistent with high permeability. These effects were significantly more intense in T5 compared to T2 and T0. The "leaky", highly vascularized capsules also showed increased astroglyosis (extreme IHC reaction for glial fibrillar acidic protein, GFAP) and the deposition of beta-amyloid peptide compared to clear capsules. A decrease in transcripts for GFAP and the amyloid precursor protein (APP) on T5 versus T2 and T0 suggested downregulation of these markers and a possible restabilization of the BBB. This is consistent with early remodeling by matrix metalloproteinases (MMP)-2 and MMP-9, suggested by their high gene expression on blue capsules at T5.We conclude that the increase of vascular permeability as a result of treatment-induced inflammation happens mainly around newly formed vessels; we also suggest the existence of a restabilization process of the BBB that follows the rapid post treatment disruption.

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AMYLOID-β PEPTIDE AND EXPRESSION OF AMYLOID PRECURSOR PROTEIN GENE (APP) ARE INDUCED BY ANTHELMINTIC TREATMENT IN PORCINE NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC), hyperendemic in Peru, is the brain infection with the larval stage (cyst) of *Taenia solium*; a main frequent clinical manifestation are seizures. This disease has been well documented clinically, but the complex host-parasite interaction and the resulting inflammation, a main factor of the severity of the symptoms, are not completely understood. Among many inflammatory markers, amyloid-beta peptides (Aβ) have been associated with neuropathological processes including glial activation, synaptic dysfunction and neuronal apoptosis. However, the role of Aβ in the neuropathology of NCC has not been explored yet the effect of anthelmintics on this molecule is unknown. We used brain sections of eight pigs naturally infected with *Taenia solium* cysts. Five animals received a single dose of 100 mg/kg praziquantel (PZQ), which is demonstrated to trigger inflammation, and three untreated pigs served as controls (T0). Three pigs were sacrificed after two days of treatment (T2) and two after five days (T5). Two hours before sacrifice, all animals were sedated and injected with Evans Blue to delineate increased permeability of the blood-brain barrier (BBB), shown to correlate with inflammation. Eighty-two cyst capsules (23 from T0, 36 from T2 and 23 from T5) were examined to evaluate the presence of Aβ in host tissue by immunohistochemistry (IHC). The relative expression of the amyloid precursor protein gene (app) was analyzed by quantitative real time PCR in 20 capsules (7 from T0, 7 from T2 and 6 from T5). We found that PZQ induced both the presence of Aβ and app expression, this was more evident when the BBB had been affected. IHC results showed similar values for T2 and T5, both being significantly higher that T0, but app expression was clearly higher for T2 than T0 and T5, indicating post-transcriptional regulation. Besides, we observed less Aβ in degenerated cysts, which suggests the possibility of a regeneration process that follows the acute inflammatory phase. Together, these findings suggest that Aβ is a component of the acute inflammatory response in NCC and that its expression is regulated at some point of the cyst degeneration.

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TAENIA SOLIUM CYSTICERCOSIS SEROCONVERSION AND SEROREVERSION CUMULATIVE INCIDENCE IN A LARGE-SCALE COMMUNITY TRIAL IN BURKINA FASO

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Cysticercosis is a debilitating yet neglected disease caused by *Taenia solium*, a zoonosis transmitted between humans and pigs. Knowledge on the disease transmission patterns is crucial to develop effective control strategies. Our objective was to estimate the seroconversion (SC) and seroreversion (SR) cumulative incidence as well as related risk factors in a large scale community randomized controlled trial in Burkina Faso. The study was conducted in all pig-raising departments of the provinces of Nayala, Boulkiemdé and Sanguié. Two villages per department meeting eligibility criteria were randomly selected and clustered sampling based on pig raising was used to select 80 concessions per village. In each concession, one household was randomly selected from which one eligible individual was randomly chosen. Sixty people were asked to consent to provide three blood samples over the course of three years in each village. Socio-demographic factors, pork cooking and eating practices and pig management practices were assessed through questionnaires. The presence of excretory secretory circulating antigens (Ag) of *T. solium* metacestodes was measured in sera using the B158/B60 enzyme-linked immunosorbent assay (ELISA). Sera were collected at baseline and 18 months follow-up from 2211 consenting individuals. The SC and SR cumulative incidences were 3.3% (95% confidence interval (CI) 2.6-4.2) and 35.8% (95% CI 24.7-48.5), respectively. Univariable analyses indicated higher SC in Boulkiemdé vs. Sanguié (4.0 vs. 2.2%, cumulative incidence ratio (CIR) = 1.8, 95% CI 1.0-3.3). While SC tended to be higher among individuals older than 40 years versus those of 6-17 years (4.3

vs. 2.5%, CIR = 1.7, 95% CI 1.0-3.1), SR was much lower in the older group (18.9 vs. 80.0%, RR = 0.2, 95% CI 0.1-0.5). Lastly, SC was higher in individuals eating pork at the village market compared to those never eating pork (4.7 vs. 2.0%, RR = 2.4, 95% CI 1.1-5.2). This is the largest cohort study of cysticercosis SC and SR ever conducted in West Africa. Multivariable and hierarchical analyses will be conducted to explore the impact of village-level and other individual level factors.

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USE OF DIFFERENTIALLY EXPRESSED MONOCYTE GENES TO DISTINGUISH BETWEEN NEUROCYSTICERCOSIS-ASSOCIATED EPILEPSY AND IDIOPATHIC EPILEPSY

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Neurocysticercosis (NCC) is a parasitic infection of the brain that accounts for 34% of active epilepsy cases in Vellore district, India. New diagnostic methods are needed due to the high cost of brain imaging and because antibody detection misses 34% of NCC patients.. We conducted a cross-sectional study of patients aged 18 to 51 years seeking care at the Dept. of Neurological Sciences, Christian Medical College and Hospital, Vellore between Jan. 2013 and Oct. 2014. Subjects were categorized as patients diagnosed with NCC-associated epilepsy with at least one seizure in the past 7 months (Group 1, n=76); recovered NCC (RNCC) patients without seizures and brain lesions for at least two years (Group 2, n=10); patients with epilepsy with at least one seizure in the past 7 months without any structural brain lesion on imaging (Group 3, n=29); patients who had a normal brain CT scan or MRI, no epilepsy or head trauma (Group 4, n=17). Groups 3 and 4 were negative for antigens or antibodies to T solium larvae in serum. Group 1 was sub-divided into those with Solitary Cysticercus Granuloma (SCG, n=29), single calcified cysts (SCC, n=20) and multiple cysts (MNCC, n=27). We used mRNA arrays of CD14+ blood monocytes from 6, 6 and 4 patients in Groups 1, 3 and 4, respectively, to identify differentially expressed genes linked to inflammation, host defenses or central nervous system processes. We identified 15 genes including GTPase's (4), and genes linked to immune regulation (4) enzymes with role in signaling (3), neurogenesis (1), and other immune function (3). Expression of these genes was measured by qPCR in all participants and fold-change in NCC cases (Groups 1-3)/Control (Group 4) was calculated. Highest expression levels were observed in patients with NCC-associated epilepsy, followed by RNCC and finally by those with idiopathic epilepsy. Expression levels of 7 of 15 genes differed among NCC patients with different types of brain lesions with expression decreasing as lesions became calcified. In contrast, one gene (RAP1A), increased as lesions calcified. This study suggests measuring expression of key blood monocytes genes may be useful to diagnose and stage NCC.

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VENTRICULAR NEUROCYSTICERCOSIS IN THE UNITED STATES: TREATMENT, COMPLICATIONS AND OUTCOME IN A TERTIARY REFERRAL CENTER

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Ventricular involvement in neurocysticercosis (NCC) has distinct clinical presentations, complications and treatments. Here we review the clinical course of 21 patients with one or more ventricular cysts referred to the National Institutes of Health (NIH) over 31 years. 21 patients with one or more ventricular cysts had a median age of 29.6 yr. (range 22.4-52.6), 52.4% male and the median follow up was 3.1 yr. (range 0.12-27.7). Most of the patients presented with a single cyst (19/21, 90.5%); two patients had 2 and 3 cysts, respectively. The 4th ventricle was involved alone in 12/21(57.1%) persons but a single cyst occupied more than one ventricle, migrated or there were multiple cysts in 16/24 (66.7%). Most of the patients presented with one or more additional manifestations of NCC, 5/21 (23.8%) with viable/degenerating parenchymal cysts, 9/21 (42.9%) with parenchymal calcification(s), and 8/21 (38.1%) with subarachnoid cysts. Only 4 had a single 4th ventricular cyst without any other accompanying types of NCC. Most of the symptoms were due to acute or chronic hydrocephalus (76.1%) including headache, vomiting, nausea, syncope, confusion, dizziness, coma, and vision disturbance, in decreasing order. A majority of patients were initially treated with cyst removal and/or shunt placement before referral to NIH. Patients with non-resectable cysts were treated medically. A number of complications were noted, mostly related to surgery prior to referral and to residual long-lasting symptoms.Of the 6 persons with only ventricular cysts and/or calcifications, 4 had sufficient follow-up and serial evaluations to assess if resolution occurred. Negative or decreasing cestode antigen levels in the CSF predicted resolution after cyst removal without the need for cysticidal treatment. Low WBCs counts in the CSF mostly corroborated the cestode antigen findings but, in one case, interpretation was complicated by the use of corticosteroids and presence of an infected shunt. Careful follow up of patients with surgically removed ventricular cysts, allows determination of the need for treatment.

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RISK FACTORS FOR SEIZURE RECURRENCE AFTER SUCCESSFUL ANTIPARASITIC TREATMENT IN PARENCHYMAL NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC), caused by the larval stage of the pork tapeworm *Taenia solium*, is a major risk factor for seizures worldwide. In this retrospective cohort analysis based on two randomized clinical trials in which patients had a systematic post treatment follow up of 18 months, patients in whom all viable cysts resolved after antiparasitic treatment were identified to assess risk factors for seizure recurrence from 6 to 18 months after treatment onset. Out of 188 patients, 85 (45.2%) had complete resolution of viable cysts demonstrated on follow up MRI at 6 months. Eight (9.4%) of these patients had at least one seizure between 6 and 18 months after treatment. A seizure in the initial month after the onset of antiparasitic treatment (present in 30 cases) was associated with seizure relapse between months 6 and 18 (6/30, 20% versus 2/55, 4%, relative risk 5.6, 95% confidence interval 1.2-25.6, p=0.02). Similarly, a seizure in the initial two months or in the initial 6 months after treatment onset were associated with further seizure episodes in the same period (2-months: 7/33, 21.2% versus 1/52, 1.9%, relative risk 11.0, 95% confidence interval 1.4-85.6, p=0.003; 6 months: 8/36, 22.2% versus 0/49, 0%, risk reduction 0.22, 95% confidence interval 0.09-0.36, p<0.001). The risk of seizure relapse was also significantly higher in patients with a history of previous courses of antiparasitic treatment (RR 6.7, 95% confidence interval 1.6-29.3, p=0.03), but no significant differences in risk of seizure relapse were found in regards to their length of seizure history, number of seizure episodes in the 12 months before antiparasitic treatment, number of cysticercotic lesions, presence of calcifications, proportion of lesions with inflammation at baseline, or length of antiepileptic drug treatment. Our results show that early post-treatment seizures will be associated with further seizure relapse in a subgroup of NCC patients. Enhanced seizure control in the first months should be considered to reduce this risk.

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FUNCTIONAL ANALYSIS OF DIVERGENT GPCR-LIKE PROTEINS DURING *PLASMODIUM CHABAUDI* BLOOD-STAGE DEVELOPMENT

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Whilst malaria infection results in a complex range of responses and disease in the host, the parasite must respond in turn to changing host conditions to optimise its infectivity and transmission capacity. The components of parasite signaling pathways, therefore, have great potential as anti-malarial therapeutic targets. Signaling pathways operating through G-Protein Coupled Receptors (GPCR) are the best-established class of therapeutic targets and, although almost nothing is known about the identities of *Plasmodium*-encoded components of GPCR signalling pathways, chemical library screens and experimental approaches suggest that they operate during *Plasmodium* blood-stage development. We have therefore investigated the function of two *Plasmodium* proteins that are members of ancient and divergent GPCR families using the rodent *in vivo* malaria model, *P. chabaudi.* Gene deletion and mutation has revealed that *Plasmodium* GPCR-like genes play a role in parasite egress and invasion and in host-parasite interactions necessary for the establishment of both acute and chronic infection. The function of GPCR-like family members in regulating the signalling pathways involved in these processes will be discussed.

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A NOVEL POPULATION OF TCRαβ-EXPRESSING CD11BHIGHCD14+F4/80+ MACROPHAGES IS INDUCED BY *PLASMODIUM BERGHEI* ANKA MURINE MALARIA

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Macrophages are equipped with a wide array of invariant receptors that facilitate their phagocytic function and the regulation of inflammation. In malaria, macrophages mediate both immune protective (parasite clearance) and pathogenic (cerebral malaria and severe malaria anemia) processes. Using the virulent asexual stage of the *Plasmodium berghei* ANKA (*Pb-A*) parasite which causes experimental cerebral malaria in C57BL/6 mice and severe anemia in Balb/c mice, we have identified a novel population of $CD11b^{high}CD14^{+}F4/80^{+}$ macrophages that express TCRαβ during malaria infection. This population expands rapidly during a *Pb-A* infection and preferentially sequesters in the brain during ECM. Proliferation of malaria specific TCRαβ-expressing macrophages requires a threshold level of parasite burden and optimal expression of TCRαβ on $CD11b^{high}$ cells requires coexpression of CD14 and F4/80. Furthermore, in depth flow cytometric analysis demonstrates that these unique TCRαβ-expressing macrophages are $CD3^{-}CD4^{-}CD8^{-}$ and the Vβ TCR repertoire of macrophages is distinctly different from conventional T cells during *Pb-A* infection. Identification of this unusual macrophage population that uses combinatorial TCRαβ expands our knowledge of macrophage biology during malaria and adds another layer of complexity to malaria immunology while providing new considerations in vaccine and drug design.

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PLASMODIUM FALCIPARUM PHISTC PROTEINS ARE REQUIRED FOR ANTIGEN DELIVERY TO THE INFECTED ERYTHROCYTE SURFACE

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Plasmodium falciparum extensively remodels its host cell to mediate nutrient exchange and avoid immune and splenic pressure during blood stage infection. This remodeling includes changes to the adhesive and biophysical characteristics of infected cells and is mediated by exported parasite proteins. Here, we investigate the role of *Plasmodium* PHISTc proteins in erythrocyte remodeling. PHISTc proteins are exported and contain a helical core domain shared by all PHIST paralogs. They are conserved across primate malarias, and several are expressed in both asexual and sexual blood stages, suggesting conserved function in host pathogen interactions. While several proteins from the PHISTb sub-class have a role in cellular rigidity or cytoskeletal architecture, little is known about the function of PHISTc proteins. To investigate the role of PHISTc proteins in asexual and sexual stage remodeling, we knocked out 9 of the 16 *P. falciparum* paralogs in the reference line 3D7 and in a second line, CS2. Flow cytometry showed that 6 of the 9 PHISTc knock outs have decreased asexual surface reactivity to serum from malaria patients, suggesting that they are required for efficient surface antigen trafficking. Of these 6 PHISTc knockouts with decreased surface antigen display, 2 showed a complete absence of the VAR2CSA PfEMP1 variant at the erythrocyte surface by flow cytometry and immunofluorescence microscopy while 2 others showed normal VAR2CSA display. These data suggest differential specificity for surface antigen delivery between PHISTc proteins. Finally, microsphere filtration showed that none of the 9 PHISTc knock outs affects cellular rigidity of asexual stages, and immunofluorescence microscopy showed that PHISTc disruption also does not affect trafficking of knob or Maurer's clefts markers. Altogether our observations suggest that PHISTc proteins, in contrast to PHISTb proteins, play a specialized role in antigen delivery to the host cell surface without drastically altering cellular architecture. We hypothesize that each PHISTc protein may differentially contribute to the trafficking of specific antigen families or PfEMP1 variants.

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EXPLORING THE ROLE OF *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN 1 IN INVASION OF DUFFY-NULL AFRICANS

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The ability of the malaria parasite *Plasmodium vivax* to invade red blood cells (RBCs) is dependent on the expression of the Duffy blood group antigen on RBCs. Consequently, Africans who are null for the Duffy antigen are not susceptible to *P. vivax* infections. Recently, *P. vivax* infections in Duffy-null Africans have been documented, raising the possibility that *P. vivax*, a virulent infection in other parts of the world, may expand malarial disease in Africa. In our study, we have identified two Duffy-null Ethiopians infected with *P. vivax*. For invasion, *P. vivax* binds the Duffy blood group antigen through its Duffy binding ligand 1 (PvDBP1). Previously several mutations were observed in PvDBP1 from Madagascar, India, and Brazil and we identified unique mutations in PvDBP1 in Duffy-

null Ethiopians by sequencing. We aimed at understanding whether the mutations in PvDBP1 results in binding to another receptor on Duffy-null RBCs. We determined PvDBP1 from these parasites failed to bind Duffy-null RBCs, but bound strongly to Duffy-positive RBCs, indicating that mutations in DBP1 did not account for the ability of *P. vivax* to infect Duffy-null Africans. Interestingly, by real-time quantitative PCR we identified three and eight copies of PvDBP1 in the two Duffy-null Ethiopians suggesting that it may be selected to bind low copy number of Duffy blood group antigen if expressed on Duffy null RBCs or another new receptor on the RBCs by increasing its gene expression. Moreover, Sal I *P. vivax* invades Squirrel monkeys despite the failure of PvDBP1 binding to squirrel RBCs. It is surprising to know that despite the high similarity between the Duffy blood group antigens from Squirrel, Aotus monkeys and humans, Sal I PvDBP1 does not bind Squirrel monkey erythrocytes. Furthermore, we determined *P. vivax* DBP1 from India and Brazil bound squirrel monkey RBCs as strongly as Aotus RBCs. Therefore, Sal I *P. vivax* infects Squirrel monkeys in the absence of PvDBP1 binding to Squirrel monkey RBCs. We conclude that *P. vivax* Sal I and perhaps *P. vivax* in Duffy null patients may have adapted to use new ligand-receptor pairs for invasion.

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY PREVALENCE: GENETIC VARIANTS AND THEIR INFLUENCE ON HEMOLYTIC EFFECT IN MALARIA ENDEMIC AREAS OF COLOMBIA

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Glucose 6-phosphate dehydrogenase (G6PD) is an enzyme involved in prevention of cellular oxidative damage, particularly protecting erythrocytes from hemolysis. An estimated 400 million people present variable degrees of inherited G6PD deficiency (G6PDd) which puts them at risk for developing hemolysis triggered by several risk factors including certain foods and multiple drugs including Primaquine (PQ). Intensification of malaria control programs worldwide are recommending a more extensive use of PQ and related drugs in populations with different levels of G6PDd prevalence. The aim of this study is to assess the prevalence of G6PDd in representative malaria endemic areas of Colombia and the influence of PQ administration on the induction of hemolysis. A total of 426 volunteers from Buenaventura, Tumaco, Tierralta and Quibdó were evaluated for G6PD enzymatic activity by using a quantitative G6PD test and a subset of samples were analyzed by PCR-RFLP to determine the frequency of the three most common G6PD genotypic variants: A-, A+ and Mediterranean. Preliminary results indicate a high frequency of G6PD A- genotype, followed by A+ genotype. A total of 28 individuals (6.56%) displayed either severe or intermediate G6PDd. The highest prevalence (3.51%) was found in Buenaventura, whereas G6PDd prevalence was lower (<1%) in Tierralta and Quibdó. G6PD A alleles were the most frequent (15.23%) particularly in Buenaventura and Tumaco. In order to determine the hemolytic effect of PQ administration for treatment of *P.vivax* infections, in a second phase, individuals attending the malaria control program are being studied. Blood samples from *P.vivax* malaria patients are being collected over a period of three weeks after initiation of PQ treatment and are being analyzed to assess hematologic parameters such as hemoglobin, hematocrit, and bilirubin as well as G6PD phenotype and genotype. Final results of these studies will be presented. Assessment of G6PDd prevalence in malaria endemic areas is crucial in view of possible mass drug administration (MDA) for malaria elimination in these regions.

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ACUTE KIDNEY INJURY IS COMMON IN UGANDAN CHILDREN WITH SEVERE MALARIA, AND STRONGLY ASSOCIATED WITH SEQUESTERED PARASITE BIOMASS AND MORTALITY

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Acute kidney injury (AKI) is a common complication in severe malaria that portends adverse clinical outcomes. A major challenge in assessing AKI relates to defining baseline kidney function. A number of approaches have been evaluated in pediatric cohorts, but none have been validated in African children. This study was performed at Mulago Hospital, Kampala, Uganda. Children with cerebral malaria (CM), severe malaria anemia (SMA), or community children (CC) were enrolled if they were between 18 months and 12 years of age. In this study we validate an approach to estimate baseline renal function in children assuming a normal glomerular filtration rate (GFR) of 120mL/min/1.73m2 to back-calculate creatinine (Cr) using the Schwartz equation. We compared the estimated baseline Cr (eCr) values to a population-derived normal curve of Cr for height using CC (n=169). The eCr calculated a GFR of 120mL/min/1.73m2 correlated very well with values derived from the reference population (R2=0.997, p<0.0001). AKI was defined using the Kidney Disease: Improving Global Outcomes (KDIGO) classification where a 1.5x increase in baseline Cr constituted AKI. Rates of AKI classification using the two approaches were identical with 39.5% of children with CM (n=99/257), and 21.0% of children with SMA (n=46/219) meeting a definition of AKI. AKI was associated with the sequestered parasite biomass in children with CM and SMA, p<0.0001 and p=0.005 respectively. In children with CM, AKI was associated with increased odds of in-hospital and all-cause 24-month mortality (odds ratio, 95% confidence interval: in-hospital mortality, 1.59, 1.04-2.45, p=0.009; 24 month mortality, 1.69, 1.09-2.61, p=0.003). This study validates a GFR of 120mL/min/1.73m2 as an appropriate estimate of baseline renal function in Ugandan children. AKI was strongly associated with sequestered, but not circulating, parasite biomass in children with both SMA and CM. In children with CM, AKI was also strongly associated with increased short- and long-term mortality. Interventions that decrease sequestration should be studied for their potential to reduce AKI and mortality in children with CM.

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PFHRP2 PERSISTENCE IN "ONCE INFECTED RBC" ENABLES A RAPID PREDICTION OF POST-ARTESUNATE DELAYED HEMOLYSIS

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Artesunate rapidly cures patients with severe malaria but frequently induces anemic episodes called Post-Artesunate Delayed Hemolysis (PADH) for which a simple predictive method is urgently needed. The concentration of "once-infected" red blood cells - appearing when

artesunate-exposed parasites are expelled from their host RBC by pitting - is predictive of subsequent PADH. In 103 French travellers patients, we observed that *Plasmodium falciparum* Histidine-Rich Protein 2 (HRP2) persists in the whole blood (not plasma) of artesunate-treated patients at significantly higher levels than quinine-treated patients (p=0.035). This HRP2 persistence was also observed in 70 Bengladesh patients. Using an optimized membrane permeation method, HRP2 was observed by immunofluorescence, Western blot and electron microscopy to persist in once-infected red cells from artesunate-treated patients. HRP2 deposition followed a membrane-bound pattern similar to that of Ring-Erythrocyte Surface Antigen (RESA), the conventional marker of these cells. Based on these observations, we developed a semi-quantitative titration method using a widely available HRP2-based RDT. Positivity of this RDT using a 1:500 dilution of whole blood collected after parasite clearance (2-4 days after start of treatment) predicted subsequent PADH with 93% sensitivity and 74% specificity. These results immediately open the way to the adaptation and adoption of existing HRP2-based malaria RDTs for cheap, bed-side prediction of PADH several days before it occurs.

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EVALUATE MOSQUITO NETS FASTER AND CHEAPER: RESULTS FOR PUBLIC HEALTH INTEREST

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The World Health Organization (WHO) promotes the use of long-lasting insecticidal nets (LLINs) to prevent malaria and also advises national programs to evaluate insecticidal activity on nets. To measure insecticidal activity, the WHO guidance mentions the use of a cone test, which needs 100 female mosquitoes per tested net. However, it requires a major and steady effort to produce thousands of insecticide-susceptible female mosquitoes, involving also expensive materials. The aim of the present study is to improve LLIN bio-efficacy testing by reducing cost and efforts while guaranteeing the accuracy of results. We evaluated two alternative methods to fulfill this objective: reducing the number of mosquitoes and evaluating a mosquito-free method. First, we compared the use of one, two, three or four cones (i.e. 25, 50, 75 or 100 mosquitoes) on each piece of LLIN with its Bayesian probability to be a valid LLIN. The result showed that using two cones has a limited impact on accuracy (93%) as compared with actual standard of four cones (94%). In a series of several LLINs, the average error in the measured proportion of valid LLINs was <1%. This result shows that it is possible to halve the time of lab processes without loss of accuracy for Public Health recommendations. Second, we evaluated a Colorimetric Field Test (CFT), a chemical method to measure surface levels of insecticide on LLINs. From three brand of LLINs collected in Madagascar, cut off values corresponding to the threshold value of 80% mortality with bio-assay test were determined at 0.35 µg, 0.60 µg and 0.07 µg per sample to consider a LLIN as 'good net'. The results showed 92% sensitivity and 100% specificity, demonstrating that CFT is an excellent tool for assessing the residual activity of the insecticide. By adopting one of these methods, one could save much time and money to make a faster and cheaper decision for malaria control programs. A decision that could save human lives!

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INTERROGATING MOSQUITO-PATHOGEN COMMUNITIES USING HIGH-THROUGHPUT MICROFLUIDICS

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Eliminating mosquito-borne diseases requires intimate knowledge of the ecology of vectors. Such knowledge can for instance be used to design effective vector control strategies, or to understand the eco-evolutionary processes that drive range expansions of vectors and pathogens. Current techniques in vector ecology, such as human landing catches or chemically baited traps, are very labor intensive and therefore severely limited in throughput. These limitations prevent the detailed interrogation of mosquito-pathogen communities in the field. We demonstrate a low-cost automated screening tool that enables dissection-free, high-throughput molecular analysis of individual vectors and their pathogens. We exploit the fact that mosquitoes transmit pathogens by expectorating saliva to autonomously collect saliva droplets resulting from single mosquito bites. Multiple cues (e.g. temperature, odorants, texture) are integrated on a microfabricated substrate mimicking human skin, the substrate is designed to maximize its attractiveness to mosquitoes and induce them to bite, thereby depositing saliva. We present behavioral data extracted from laboratory experiments that allow us to quantitatively assess the interaction of mosquitoes with the device. The use of high-throughput microfluidics enables us to perform small volume biochemical analyses on a huge number of pico-to-nanoliter saliva samples in parallel, greatly reducing reagent cost and processing time. We implement multiplexed microfluidic assays that enable the simultaneous characterization of the biting mosquito's genetic make-up and its pathogens. This platform provides us with a means of high-throughput, high-resolution sampling of individual insects in field and laboratory settings. Large scale application of this tool in public health surveillance may provide early warnings for epidemics, detect the emergence of drug resistance, and track the spread of emerging infectious diseases.

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A PROMISING NEW MODE OF ACTION CHEMISTRY INDOOR RESIDUAL SPRAY PRODUCT TO CONTROL RESISTANT MOSQUITOES

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Widespread insecticide resistance of Anopheline mosquitoes to pyrethroids has dramatically reduced their effectiveness and as a consequence their use in indoor residual spray (IRS) programs has declined dramatically. Currently there are only three other mode of action insecticides recommended by the World Health Organization that are currently in use as IRS adulticides: carbamates, organophosphates and organochlorines, all of which also have cases of resistance reported. A new mode of action IRS chemistry is therefore urgently needed to control resistant populations and also to help prolong the life of existing IRS chemistry by rotating applications over time between different classes. SumiShield™ 50 WG is an indoor residual spray product based on the neonicitinoid insecticide clothianidin that has previously not been used in vector control. Initial laboratory studies have shown excellent residual activity of this water dispersible granule (WG) formulation on typical indoor surfaces such as mud, wood and cement. Experimental hut and semi field studies have also been conducted that confirm these initial findings and have shown residual activity of over 6 months against both susceptible and resistant strains. These findings are discussed in detail. This clothianidin based IRS product is currently undergoing evaluation by the World Health Pesticide Evaluation Scheme (WHOPES) in both experimental huts and also in village scale trials. Introducing an alternative mode of action chemistry to the marketplace will allow use of IRS products to be rotated and thus help facilitate the implementation of the WHO Global Plan for Insecticide Resistance Management (GPRIM). When used alongside other interventions such as bed nets and other modes of action IRS products

SumiShield 50 WG will be a valuable tool to help control both susceptible and resistant mosquitoes and bring us closer to the goal of eliminating and ultimately eradicating malaria.

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SHAZAM FOR MOSQUITOES : CROWDSOURCING VECTOR SURVEILLANCE BY USING MOBILE PHONES AS ACOUSTIC SENSORS

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One of the most ubiquitous, powerful and easily accessible data collection tools that we possess today is the mobile phone. Here we show that mobile phones can be harnessed to identify insects around us through acoustic measurements. We demonstrate that a variety of mobile phones, from recent smart phones to the feature-phone models of the 1990s, can be used to record sufficiently sensitive signals for the acoustic identification of individual insects. Through signal processing analyses, we create an "acoustic fingerprint" for disease vectors such as mosquitoes, which can be used to infer its species and physiological characteristics like sex and blood-feeding status. We outline a citizen science initiative to create an open database of acoustic signatures, with insect recordings from all over the world being processed for crowd-sourced, real time surveillance of vector ecology. This technique makes it possible to collect vector surveillance data at extremely high spatio-temporal resolutions, which can help plan disease control strategies, monitor invasive species and gauge infection risks for vector-borne diseases.

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AUTOCIDAL MOSQUITO CONTROL: ALLOWING MOSQUITOES TO HELP US WITH OUR WORK

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The ongoing problem of mosquito borne disease provides an impetus to develop additional methods for the control of invasive mosquito species and against the globalization of mosquito-vectored pathogens. In addition to the development of new active ingredients, there is need also to develop additional methods for delivering pesticides. Autocidal methods rely on the use of mosquitoes to 'self-deliver' pesticides and may provide a useful compliment to traditional application methods. Here, the results of recent field trials will be presented. The trials are based on the release of male mosquitoes that have been either 1) infected with a naturally-occurring bacterium "Wolbachia" to cause sterility in a targeted population or 2) dusted with pyriproxyfen, which is a powerful inhibitor of immature mosquito development. The Wolbachia method is non-GMO and categorized by the EPA as a microbial biopesticide. The Wolbachia method is species specific and has been developed for multiple mosquito species, including *Aedes albopictus*, *Ae. aegypti* and *Anopheles stephensi*. The pyriproxyfen dusting approach can be used alone, or combined with classical Sterile Insect Technique, Wolbachia and GMO approaches, to increase the overall impact of the introduced male mosquitoes. The different approaches will be discussed and contrasted, and their relevance to different mosquito control contexts, including areal delivery, will be discussed. The results of recent field trials will be summarized.

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TRANSGENIC INSECT KILLING FUNGUS BETTER KILLS INSECTICIDE-RESISTANT, MALARIA-VECTOR MOSQUITOES

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The continued success of malaria control efforts requires the development, study and implementation of new technologies. Here we present a significant advancement in the development of insect killing fungi as a means of mosquito biocontrol. We have combined the natural abilities of *Metarhizium* spp. fungi and the field of arthropod-derived toxins to engineer a highly specific and potent pathogen of mosquitoes. Our studies show significant improvements in the rate of mosquito mortality due to the transgene in both insecticide-susceptible and wild-caught, insecticide-resistant populations. We further discovered the enhanced ability of the transgenic fungus to decrease a critical aspect of mosquito behavior for the spread of malaria (blood feeding behavior). In only 5 days, the transgenic fungus not only decreases disease transmission in mosquitoes faster than the wild-type fungus, but it decreases blood feeding to a greater degree. This research characterizes our transgenic entomopathogen as an effective and rapid mosquito control technology, which can be readily applied to mitigate risks involved with existing insecticide-based control methods.

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COMPARING THE EFFICACY OF INSECTICIDE MIXTURE AND COMBINATION STRATEGIES FOR IMPROVED CONTROL AND MANAGEMENT OF PYRETHROID RESISTANT MALARIA VECTORS IN SOUTHERN BENIN, WEST AFRICA

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The Global Plan for Insecticide Resistance Management (GPIRM) recommends the use of a combination/mixture of unrelated insecticides against insecticide resistant malaria vectors for improving control and managing resistance. This concept can be applied by using insecticide mixtures on mosquito bed nets, co-formulated insecticide mixtures for indoor residual spraying (IRS) or deploying non-pyrethroid IRS together with pyrethroid bed nets. The uptake of these strategies at national levels has been slow due to lack of effective new mixture/combination tools and insufficient evidence on efficacy to guide decision making. Mixtures on bed nets or IRS being single interventions could be cost effective and more desirable than the combined intervention approach if comparable levels of impact can be demonstrated in terms of their ability to improve vector control and manage resistance. The current study compared the efficacy of a newly developed chlorfenapyr (a pyrolle) and alphacypermethrin (a pyrethroid) mixture long-lasting bed net (Interceptor G2) to an IRS mixture of chlorfenapyr and alphacypermethrin and a combined chlorfenapyr IRS and alphacyperthrin long-lasting net (Interceptor 1) intervention in experimental huts against wild pyrethroid resistant malaria vectors in Cove, Benin. Mortality rates were very low with alphacypermethrin IRS alone (5%) and Interceptor 1 alone (24%) owing to high levels of pyrethroid resistance in the vector population. Mortality with the mixture IRS (42%) was unexpectedly lower than IRS with chlorfenapyr alone (51%) ($P<0.005$). The mixture net (Interceptor G2) and the combined IRS and bed net approach provided the highest mortality rates and these were similar between both treatments (75% vs. 69% respectively, $P>0.05$). Blood feeding rates were also significantly reduced with these treatments compared to the control and the mixture IRS ($P>0.05$). The results demonstrate that Interceptor G2 could be a more cost-effective and

reliable strategy for improving the control of pyrethroid resistant malaria vectors and managing resistance compared to the combined intervention approach and the mixture IRS.

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LYMPHATIC FILARIASIS ELIMINATION IN AMERICAN SAMOA: EVALUATION OF MOLECULAR XENOMONITORING AS A SURVEILLANCE TOOL IN THE ENDGAME

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The Global Programme to Eliminate Lymphatic Filariasis (GPELF) has made significant progress toward interrupting transmission of lymphatic filariasis (LF) through mass drug administration (MDA). Operational challenges in defining endpoints of elimination programs have been identified, including the need to determine appropriate post-MDA surveillance strategies. As humans are the only reservoirs of LF parasites, one such strategy is molecular xenomonitoring (MX), the detection of filarial DNA in mosquitoes using molecular methods (PCR), to provide an indirect indicator of infected persons nearby. MX could potentially be used to evaluate program success, provide support for decisions to stop MDA, and conduct post-MDA surveillance. American Samoa has successfully completed MDA and passed WHO recommended Transmission Assessment Surveys in 2011 and 2015, but recent studies using spatial analysis of antigen and antibody prevalence in adults (aged ≥18 years) and entomological surveys showed evidence of possible ongoing transmission. This study evaluated MX as a surveillance tool in American Samoa by linking village-level results of the recent human and mosquito studies. Of 32 villages, seropositive persons for Og4C3 antigen were identified in 11 (34.4%), Wb123 antibody in 18 (56.3%) and Bm14 antibody in 27 (84.4%) of villages. Village-level seroprevalence ranged from 0%-33%, 0%-67% and 0%-100% for Og4C3, Wb123 and Bm14 respectively. PCR-positive *Aedes polynesiensis* mosquitoes were found in 15 (47%) villages, and their presence was associated with a significantly higher probability of seropositive persons for Og4C3 (67% vs 6%) and Wb123 (87% vs 29%), but not Bm14. In villages with seropositive persons for Og4C3 and Wb123, PCR-positive *A. polynesiensis* were found in 91% and 72% respectively. In villages without Og4C3-positive persons, PCR-positive *A. polynesiensis* were absent in 94%. Our study provides promising evidence to support the potential usefulness of MX in post-MDA surveillance in an *Aedes* transmission area in the Pacific Island setting, and to predict sub-national areas where LF transmission may be continuing.

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FILARIASES IN GABON: EMPIRIC ASSESSMENTS REDEFINE DISTRIBUTION AND TREATMENT STRATEGIES FOR ONCHOCERCIASIS AND LOIASIS

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Previous surveys for Rapid Epidemiological Mapping of Onchocerciasis (REMO) in Gabon predicted very low prevalence of onchocerciasis (0 – 5%) across the country, whereas Rapid Assessment for Loiasis (RAPLOA) predicted more than 40% prevalence in the entire country. Ivermectin was never given in Gabon due to the low onchocerciasis prevalence and the considerable risk of serious adverse events (SAE) to ivermectin in highly-prevalent *Loa loa* endemic areas where individuals with *Loa loa* infections with intensities of >30,000 mf/ml may reside. Due to the new global target to eliminate onchocerciasis, there is an urgent need to determine which treatment intervention is required to eliminate the disease from Gabon. Therefore, in 2014-2015, 7,108 individuals in 93 communities in 34 districts were tested for onchocerciasis by skin snip and/or Ov16, and 10,214 individuals in 176 communities in 43 districts were tested by blood smear to detect *L. loa* microfilaremia. Prevalence of onchocerciasis was found to be much higher in some communities than predicted by REMO; 9% of districts were hyper-endemic (at least one community > 60% infected) and 12% of districts were meso-endemic (at least one community 40 - 60% infected). In contrast, *L. loa* prevalence was lower than predicted by RAPLOA; 82% of individuals tested were negative, and in one district the maximum prevalence found in any community was 0% despite a RAPLOA prediction of >40%. Of 93 communities tested for onchocerciasis, 67 communities (72%) were endemic (any positive results from skin snip or Ov16). All 67 communities had some *L. loa* prevalence (ranging from 2% to 51%), but, importantly, 31 communities (46%) had no individuals with high intensity (30,000 mf/mL+) infection. Our results have shown that it is possible to find areas with very little *L. loa* infection and high onchocerciasis burden where treatment with ivermectin could be appropriate. They also emphasize the importance of empiric assessments to refine our understanding of the distribution of these two diseases in Central Africa and guide treatment interventions to achieve onchocerciasis elimination.

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HIGH PREVALENCE OF EPILEPSY IN ONCHOCERCIASIS ENDEMIC REGIONS IN THE DEMOCRATIC REPUBLIC OF THE CONGO (DRC)

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Between 2014 and 2016, we conducted epilepsy prevalence surveys in 61 villages in onchocerciasis endemic areas in the DRC. A case control study was performed in Titule (Bas-Uélé), Salambongo (Tshopo) and Draju (Ituri). Cases were patients with active convulsive epilepsy and controls were age matched persons without epilepsy randomly selected from the same village. A high prevalence of epilepsy was observed in villages located closely to Simulidae (blackfly) infested rivers: 2.3-6.4% in Bas-Uélé, 1.5-6.0% in Tshopo, and 3.0-5.6% in Ituri. Epilepsy cases showed a marked spatial pattern with clustering of cases occurring within and between adjacent households. Individual risk for epilepsy was found to be associated with living close to the river. Peak onset of epilepsy was around the age of 14-15. Nodding syndrome was not observed but adolescents with epilepsy and with severe stunting and without secondary external signs of sexual development were observed in several villages. Phenobarbital was the anti-epileptic drug most frequently used but rarely continuously. In villages where Ivermectin was distributed for at least 10 years, no difference in the presence of *Onchocerca volvulus* (OV) DNA in the skin was observed between cases and controls. On the other hand in Draju, where Ivermectin was never distributed, OV microfilaria were observed in skin snips of 55.9% (33/59) of epilepsy cases compared to 29% (20/69) of controls ($p = 0.002$); mean density of microfilaria (Mf) in skin snips of cases was 33.6 parasites/mg skin compared to 3.8 parasites/mg skin in controls ($p = 0.002$); and 45.8 % (27/59) of cases had OV16 antibodies compared to 26.1% (18/69) of controls ($p = 0.002$). A quantitative real-time PCR assay showed that the amount of Wolbachia ftsZ gene (bacterial OV endosymbiont) in skin snips was significantly higher in cases than in controls ($p < 0.01$). In conclusion, the prevalence of

epilepsy in villages in onchocerciasis endemic areas in the DRC was 2-10 times higher than in non-onchocerciasis endemic regions in Africa. Our study confirms that OV infestation is associated with epilepsy.

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SIMULATING THE EFFECT OF EVALUATION UNIT SIZE IN DETERMINING ELIGIBILITY TO STOP MASS DRUG ADMINISTRATION FOR LYMPHATIC FILARIASIS IN HAITI

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The Transmission Assessment Survey (TAS) was designed as a decision-making tool for program managers to determine when transmission of lymphatic filariasis (LF) is presumed to have reached a level low enough that it cannot be sustained even in the absence of mass drug administration (MDA). The geographic area over which a TAS is applied is referred to as an evaluation unit (EU). EUs may comprise one or multiple program implementation units (IUs) and should have no more than 2 million people. In 2015, TAS was conducted in 14 EUs in Haiti, many comprising a single IU. Of these 14 TAS, two failed and one had a borderline result (i.e., the number of positive results was equal to the critical threshold). Simulations were used to understand what the programmatic conclusions would have been had Haiti chosen to form larger EUs. Eight "combination-EUs" were formed through various groupings of existing adjacent EUs. Several simulation approaches to replicate TAS were carried out in these combination-EUs, using bootstrapping to simulate the expected data. Each approach was replicated 1000 times, with the number of "passing" and "failing" TAS results recorded. The simulations showed that when the combination-EU was comprised of discordant EUs - at least one "passing" and one "failing" - the combination-EU would pass the TAS 71% - 100% of the time, with exception of one combination-EU, where the TAS failure rate of the combination-EU was never more than 83%. Combining IUs to form large EUs is common practice, as it can result in considerable cost savings and detailed prevalence data to better inform such decisions is often lacking. Our results demonstrate the high potential for misclassification when the prevalence of LF in the combined IUs differs. Of particular concern is the risk of "passing" larger EUs that include focal areas where prevalence is high enough to be potentially self-sustaining. Our results reaffirm the approach that Haiti took in forming smaller EUs, based on historical knowledge, and suggests that in areas where such information is lacking greater detail, perhaps gathered through additional spot check sites, may be useful to inform EU creation.

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PREVALENCE OF OV16 ANTIBODIES AMONG SCHOOL-AGE CHILDREN AFTER TWENTY YEARS OF MASS TREATMENT WITH IVERMECTIN IN TOGO

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The Onchocerciasis Control Program of Togo (OCP) is in the process of shifting its focus from control to elimination of onchocerciasis. Thirty-two of Togo's 40 districts are endemic for onchocerciasis and have been receiving annual treatment with ivermectin in villages with population ≤2000 for more than 20 years, and the 15 northern most districts receive two rounds of treatment per year. Yearly epidemiological surveillance focuses on approximately 300 villages where onchocerciasis remains prevalent according to skin snip surveys. In preparation for the move to elimination, Togo's Ministry of Health conducted a survey to obtain preliminary data on the distribution of antibodies to the Ov16 protein of *Onchocerca volvulus* in school-age children outside of the areas of ongoing surveillance. The survey was integrated with an impact assessment for other neglected tropical diseases (NTD). In 2015, in each of 1126 schools serving as NTD sentinel sites, a convenience sample of 8 school-going children aged 6 to 9 years had finger-stick blood drawn for the Ov16 rapid test. Altogether, 9007 children were tested by Ov16 rapid test and 60 (0.7%) children tested positive. A map of the locations of the 60 children with positive results shows that most were from areas where onchocerciasis is believed to be low, and one child was from a district that was categorized as non-endemic according to baseline mapping and has never received mass treatment with ivermectin. The OCP is conducting follow-up visits for all 60 children to document residency and travel history, to repeat the Ov16 rapid test, and to conduct skin snip testing with treatment if indicated. While there are challenges and limitations associated with use of the Ov16 rapid test for onchocerciasis, this survey provides important information for Togo's OCP in moving toward onchocerciasis elimination in Togo.

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TOWARDS ELIMINATION OF LYMPHATIC FILARIASIS IN MALI BY 2020

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In Mali all 63 health districts (HD) were endemic for lymphatic filariasis (LF) and an initial mapping with ICT cards in 2004 revealed a national prevalence of 7.1% (from 1% in the north to 18.6% in the south). The country committed to the goal of eliminating LF as a public health problem by 2020. The implementation of mass drug administration (MDA) with albendazole and ivermectin began in areas co endemic with onchocerciasis in southern Mali in 2005, and scaled up to other endemic areas in northern Mali, reaching 100% geographical coverage in 2009. Since 2008, Mali has benefited from the support from Helen Keller International with funding from the USAID's NTD Control Program and ENVISION Project, managed by RTI International. According to World Health Organization guidelines, LF transmission assessment surveys (TAS) were conducted in districts which had had at least 5 effective rounds of MDA. The Survey Sample Builder (SSB) was used to determine sample sizes and the selection of clusters. Evaluation units (EU) were approved by the RPRG before the implementation of the surveys. By 2016, transmission assessment surveys have been conducted in 31 health districts (11 EUs) across Mali. The 31 districts surveyed included all 10 districts in the Koulikoro region, all 6 communes in the district of Bamako, 9 out of 10 districts in Sikasso region, and 6 out of 8 districts in Segou region. A community based cluster sampling (in 9 EUs) and school-based cluster sampling (2 EUs) strategy was used depending on school enrollment rates. All 11 EU surveyed passed the TAS with the number of positive cases being below the critical cut-off values determined by the SSB (18 or 20 positive cases). Currently, 49% (31/63) of the originally LF endemic districts have reached the criteria to stop MDA and evaluations are in preparation for the 13 other districts in the center and south Mali and should be completed by June 2016. Insecurity in the north has caused some MDA rounds

to be cancelled and made it impossible to carry out evaluations. If the security situation improves, Mali will be on course to achieve national LF elimination objectives by 2020.

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EMPLOYING THE NEW OV16 RAPID DIAGNOSTIC TEST (RDT) TO EVALUATE ONCHOCERCIASIS IN AFRICA

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Elimination of onchocerciasis in Africa is targeted to be achieved by 2025. Over the next decade, thousands of people will need to be tested to identify where treatment interventions are needed, when transmission interruption has been achieved, and for surveillance to verify that elimination has been attained. Nodule palpation and skin-snip assessment of microfiladermia have been the traditional diagnostic tools, but a principal limitation of both is their lack of sensitivity in low prevalence settings. Because the Ov16 ELISA test has already been proven a useful tool in the Americas and a few settings in Africa to assess the interruption of transmission of onchocerciasis, World Health Organization (WHO) guidelines now recommend Ov16 serology as a diagnostic tool for onchocerciasis elimination programs. A new, point-of-care rapid diagnostic test (RDT) which detects antibodies to the parasite antigen Ov16 has recently been developed by PATH and its partners. While benefits of the RDT include requiring no cold chain, its ease of use, and being relatively low-cost, this test still requires evaluation at scale before it can be recommended for routine programmatic use. Therefore, in a multi-country study to compare skin snip tests with the new Ov16 RDT, a total of 31,633 people were tested in 3 post-treatment settings (in Mali, Malawi, Guinea Bissau) and in 3 settings where treatment for onchocerciasis has never been given (in Gabon, Nigeria, the Democratic Republic of the Congo). In the post-treatment settings, the prevalence of onchocerciasis as diagnosed by a positive Ov16 RDT was 3.18% (95% CI: 2.74-3.62) but 0.00% when diagnosed by skin snip. In the treatment-naïve settings, the prevalence via Ov16 RDT was 3.30% (95% CI: 3.11-3.55) and by skin snip was 1.00% (95% CI: 0.90-1.10). The Ov16 RDT was found to have a higher sensitivity compared with the skin snip diagnostic for all age groups in both the pre- and post-treatment settings. It is likely that as similar experience accumulates in other studies, the Ov16 RDT will become the standard diagnostic tool in the future for monitoring all programs aiming to achieve elimination of onchocerciasis in Africa.

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A SINGLE DOSE OF TV005 ELICITS COMPLETE PROTECTION AGAINST CHALLENGE WITH THE HETEROTYPIC DENV-2, RDEN2Δ30

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Over the past several decades dengue has become hyper-endemic in all tropical and sub-tropical regions of the world. Current estimates report nearly 100 million symptomatic and 300 million asymptomatic dengue infections annually. Dengue can range from an asymptomatic or mildly symptomatic illness to one that results in bleeding diatheses, plasma leakage, and vascular collapse. The recently licensed dengue vaccine Dengvaxia™, was found to be significantly less efficacious in persons who were sero-naive to dengue at the time of vaccination and regulatory authorities, including the WHO SAGE committee, have recommended that it be administered only to children 9 years or older living in highly dengue endemic regions. Previously, we demonstrated that the live attenuated tetravalent dengue vaccine (LATV) TV003 elicited complete protection against DENV-2 challenge using our controlled dengue human infection model (DHIM). As a first assessment of the protective efficacy of our second LATV dengue vaccine candidate TV005 in sero-naïve subjects, we conducted a randomized, placebo-controlled, double-blind trial utilizing this same DHIM. Forty-eight flavivirus-naïve subjects were enrolled. On Study Day 0, 24 subjects received TV005 and 24 subjects received a placebo. Six months later, 43 subjects returned and received 1,000 PFU of the DEN2Δ30 challenge virus (22 TV005 recipients and 21 controls). The DEN2 challenge virus is a different genotype than that included in the vaccine. All 21 controls had detectable viremia following challenge and all developed rash. None of the TV005 recipients had detectable DENV-2 in the blood following challenge and none developed rash. The LATV TV005 induced complete protection against the both viremia rash. In addition, 70% of TV005 recipients demonstrated sterilizing immunity to challenge as evidenced by lack of detectable virus in the blood and a < 4-fold rise in serum neutralizing antibody to DEN2. These data demonstrate that TV005 induces strong protection in subjects who are flavivirus-naïve at the time of vaccination.

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HUMAN CD4+ T CELL RESPONSES INDUCED BY A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE PARALLEL THOSE INDUCED BY NATURAL INFECTION, IN MAGNITUDE, HLA RESTRICTION AND FINE ANTIGEN SPECIFICITY

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Four dengue virus (DENV) serotypes are responsible for a growing number of infections, making DENV the most frequent mosquito-transmitted viral disease in humans, and creating a unique challenge for vaccine development. While considerable debate exists regarding which immune mechanisms may confer protection, a hallmark of live attenuated vaccines (LAV) is their ability to induce both humoral and cellular immune memory. We previously demonstrated that DENV-specific CD8+ T cell responses elicited by live attenuated DENV vaccines resemble those elicited by natural infection. CD4+ T cells are also a key component of cellular immunity, and contribute to host protection directly through cytokine production, and indirectly by providing help for CD8+ and antibody responses. Here, we characterize for the first time CD4+ T cell responses after live attenuated dengue vaccination and compare them to responses observed in natural infection with dengue virus. PBMCs from study participants receiving the tetravalent live attenuated DENV vaccine (TV-003), developed by the U.S. National Institutes of Health were screened in IFNγ ELISPOT assays with pools of HLA matched, predicted class II binding peptides covering the entire DENV proteome. CD4+ T cell responses were detected with magnitude and breadth similar to natural dengue infection. In natural

infection and vaccines alike, DENV specific CD4+ T cells are focused dominantly focused on the capsid, NS3 and NS5 antigens, while the envelope protein is a minor target for CD4+ DENV specific T cells.

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A PHASE I CLINICAL TRIAL EVALUATING THE IMPACT OF TETRAVALENT RECOMBINANT SUBUNIT DENGUE VACCINE BOOST ADMINISTERED TO SUBJECTS WHO HAVE PREVIOUSLY BEEN VACCINATED WITH A LIVE-ATTENUATED TETRAVALENT DENGUE VACCINE

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With the increasing global burden of dengue, there remains an urgent need for dengue vaccines. The V180 vaccine candidate consists of four truncated, soluble, dengue envelope glycoproteins (DEN-80E). The vaccine has been shown to induce robust virus-neutralizing antibody responses when formulated with ISCOMATRIX™ adjuvant and administered to flavivirus-naïve volunteers in a Phase I clinical trial (NCT01477580). V180 was also tested in a Phase I clinical trial (NCT02450838) where subjects who had previously received the live-attenuated tetravalent vaccine (LATV) developed by the National Institute of Allergy and Infectious Diseases (NIAID) were administered a V180 booster dose. The study was designed to assess whether a recombinant subunit vaccine is able to boost the trivalent or tetravalent responses induced by the LATV vaccine, which have proven difficult to boost with an additional dose of the LATV itself. The study was a randomized, placebo-controlled, double-blind study of safety and immunogenicity of the V180 vaccine. Twenty subjects who had previously received one or two doses of LATV were randomized and received a single dose of V180 non-adjuvanted (N=8), V180 adjuvanted with Alhydrogel™ (N=8), or placebo (N=4). Vaccine safety (solicited and unsolicited adverse events) was assessed using a Vaccination Report Card for 28 days following vaccination. Serious adverse events were captured from the time of informed consent through the final study visit at 6 months postvaccination. Immunogenicity was assessed using a plaque reduction neutralization test at Day 0, Day 14, Day 28, and Month 6 relative to vaccination. The results of the study demonstrate that the vaccine is generally well tolerated and immunogenic in these dengue-experienced volunteers.

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TETRAVALENT DENGUE HETEROLOGOUS PRIME-BOOST VACCINATION - SAFETY, HUMORAL, AND CELL-MEDIATED IMMUNITY AT 1 AND 6 MONTHS

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Heterologous prime boost approaches have been explored as vaccination strategies in a number of infectious diseases. We studied whether sequential administration of live and inactivated tetravalent dengue vaccine candidates could improve safety and immunogenicity performance metrics. Tetravalent dengue virus live-attenuated vaccine (LAV) and tetravalent dengue virus purified inactivated vaccine (PIV) have been evaluated previously using homologous two-dose strategies. This phase 1, randomized, open-label, primary vaccination study conducted in non-dengue endemic region (Maryland, USA). Eighty subjects were enrolled into 4 heterologous prime-boost vaccination treatment groups (N=20): Group 1 = LAV (day 0), PIV (1 month); Group 2 = PIV (day 0), LAV (1 month); Group 3 = LAV (day 0), PIV (6 month); Group 4 = PIV (day 0), LAV (6 month). Subjects were followed for 6 months after vaccination. Safety (primary end point), microneutralization (MN50) antibody titers, RNAemia, and cell-medicated immunity (CMI) were assessed. At one month after the second vaccination, there were no related severe adverse events (SAEs), no related medically attended adverse events (AEs), and no grade 3 related local AEs. Grade 3 related systemic AEs occurred in small numbers (N=11), with the majority in Group 4. LAV associated rash was observed in 8 subjects. Post LAV dengue RNAemia was detected in 18%, 26%, 20%, and 61% of the subjects in groups 1, 2, 3, and 4 respectively. At one month, PIV priming followed by LAV boost generated superior MN50 antibody titers and broader seroconversion rates with no clear difference between Groups 2 and 4. One month CMI was assessed by an IFN-γ ELISpot assay and overlapping peptide pools from all four serotypes. PIV priming appeared to result in the highest magnitude of response and the most balanced median IFN-γ spot counts. These results suggest a PIV prime, LAV boost strategy against dengue has further developmental potential despite manufacturing challenges. All follow up study visits were completed in March 2016. Safety, MN50, RNAemia, and CMI at 6 months post boost dose will also be presented.

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TAKEDA'S TETRAVALENT DENGUE VACCINE (TDV) CANDIDATE PROGRESSES TO PHASE III: SAFETY AND IMMUNOGENICITY OF TDV

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Takeda's live attenuated tetravalent dengue vaccine candidate (TDV) contains a molecularly characterized dengue serotype 2 virus (TDV-2) and three recombinant viruses expressing the pre-membrane (prM) and envelope (E) structural genes for serotypes 1, 3, and 4 in the attenuated TDV-2 backbone. On the path to phase III, Takeda has investigated different formulations, routes of administration, dosage schedules and vaccine presentations, through four phase I and four phase II studies involving more than 3800 participants (adults and children in endemic and non-endemic countries). The TDV clinical program has followed the WHO guideline for dengue vaccine development. The safety and immunogenicity profile of TDV supports continued clinical development. Key data from the development program will be presented and their implications for decisions affecting the schedule and formulation will be discussed.

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CONTRIBUTIONS OF SILENT INFECTIONS TO DENGUE VIRUS TRANSMISSION

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A considerable fraction of dengue virus (DENV) infections is thought to result in either no detectable symptoms (asymptomatic) or symptoms that are sufficiently mild that they go undetected by surveillance systems (clinically inapparent). The unknown contribution of undetected infections to the transmission of DENV and other emerging mosquito-borne viruses, like Zika virus, raises questions about the effectiveness of reactive practices for detecting and responding to outbreaks. Despite estimates that 293 million people experience either asymptomatic or clinically inapparent infection each year, it has been assumed that these individuals contribute little to onward transmission. Recently, however, blood-feeding experiments with *Aedes aegypti* showed that people with asymptomatic DENV infections are capable of infecting mosquitoes. We combined those findings with models of within-host DENV dynamics and human demographic projections to: (1) quantify the net infectiousness of individuals that experience either asymptomatic or symptomatic (either clinically inapparent or clinically apparent) infections, and (2) quantify the contributions of asymptomatic and symptomatic infections to DENV force of infection, which depends not just on their infectiousness but also on their numerical prominence in a population. Our calculations indicate that individuals with asymptomatic infections have a lower net infectiousness than symptomatic infections, yet they are still capable of making appreciable contributions to DENV transmission. We estimate that approximately two-thirds of infections could result from individuals with undetected infections. Among infections that result from clinically apparent infected individuals, more than half could result from mosquitoes biting during the pre-symptomatic phase of the infection. Our findings emphasize the need to reorient current practices for responding to outbreaks of dengue and Zika viruses, to pre-emptive interventions that take account of the role of undetected infections in DENV transmission dynamics.

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PREDICTORS FOR SEVERE DENGUE: RESULTS FROM A PROSPECTIVE MULTI-CENTRE STUDY IN EIGHT COUNTRIES ACROSS ASIA AND LATIN AMERICA

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The clinical spectrum of symptomatic dengue is broad, ranging from mild febrile illness to severe disease with potentially life-threatening complications such as bleeding, organ impairment, and plasma leakage that may result in hypovolemic shock. Without an effective therapy patient outcomes depend primarily on appropriate triage and judicious use of intravenous fluids. However although the revised 2009 WHO guidelines indicate a number of warning signs to identify potentially severe cases early, the evidence base to support these recommendations is presently limited. A prospective multi-centre observational study recruiting out-patients aged ≥ 5 yrs with symptoms consistent with possible dengue within 72 hrs of fever onset is in progress in 8 countries across Asia and Latin America, aiming to enrol around 3000 participants with confirmed dengue by June 2016. A broad range of clinical and laboratory features are assessed daily during the acute illness, and at follow-up 1 week later. We aim to identify clinical and/or simple laboratory predictors of severe dengue, in particular to explore the value of repeated measurements during the febrile phase. Severe outcomes are defined according to the need for hospitalisation or intravenous fluid administration, as well as by the WHO 2009 guidelines. After assessing heterogeneity of outcomes across countries, we will investigate the predictive value of a range of candidate predictors in classical prognostic models depending on baseline covariates only, and also explore dynamic prognostic models which take into account longitudinal data. Despite heterogeneity in clinical management between sites, preliminary findings in 2254 patients with laboratory-confirmed dengue from 5 countries suggest certain parameters, eg haematological indices such as the platelet count, to be of interest. A detailed description of the disease spectrum and risk factors identified among ~3000 dengue cases will be presented, representing the most comprehensive dataset available to date. We anticipate these results will have substantial impact on future triage and management policies in dengue endemic countries.

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ACT PARTNER DRUG EROSION—EVIDENCE OF PPQ RESISTANT PARASITES FROM CAMBODIA

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With the emergence and spread of artemisinin (ART) resistance, more pressure is put on the partner drugs (e.g., piperaquine) used in ART combination therapies (ACTs). Recent data from Western Cambodia indicate that ART and piperaquine (PPQ) resistance has emerged in this region, leading to declining frontline ACT efficacy. To assess whether ART-resistant parasites also show resistance to PPQ, we obtained 157 cryopreserved isolates from Pursat and Pailin, Western Cambodia that were collected by the Tracking Resistance to Artemisinin Collaboration (TRAC). We culture-adapted 68 parasites and focused on parasites with slow clearance rates in patients and high % survival values in the ring-stage survival assay (RSA) *in vitro* – two ART-resistance phenotypes. We first exposed parasites to a single high PPQ dose (2 μM) for 72 hours and found that some parasites were able to recover. We then profiled 23 isolates by standard EC50 assays. A PPQ-resistant subset of these isolates showed a bimodal response to PPQ with increased survival under higher drug pressure, while their primary EC50 values were comparable to sensitive isolates (5 nM). This bimodal response was not due to the presence of mixed parasite populations within the isolate, as daughter clones retained the phenotype. Exposure of parasites to PPQ at consecutive 12-hour intervals throughout the lifecycle showed that later stages (schizonts) are less susceptible than earlier stage parasites. Use of available whole-genome sequencing data and independent interrogation of these PPQ-resistant isolates did not identify any of the previously-reported markers of PPQ resistance. These results confirm the existence of PPQ-resistant parasites in Cambodia. Ongoing studies aim to identify the underlying mechanism of PPQ resistance.

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ARTESUNATE-MEFLOQUINE EFFECTIVELY TREATS DIHYDROARTEMISININ-PIPERAQUINE-RESISTANT *PLASMODIUM FALCIPARUM* MALARIA IN CAMBODIA

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Artemisinin (ART) combination therapies (ACTs) are recommended worldwide for the treatment of *Plasmodium falciparum* malaria. Dihydroartemisinin-piperaquine (DHA-PPQ) was adopted as the frontline ACT in Cambodia in 2008, and was safe, tolerable, and 96-98% efficacious. However, the rapid spread of ART resistance, defined as a parasite clearance half-life >5 h following treatment, exposes a larger biomass of parasites to the unprotected partner drug – due to the short (~1 h) elimination half-life of the ART derivative, and long elimination half-life of the partner drug. We postulated that parasites in areas with ART resistance would readily develop partner drug resistance, resulting in decreased ACT efficacy. Among patients treated with DHA-PPQ in western, northern, and eastern Cambodia (in Pursat, Preah Vihear, and Ratanakiri provinces) in 2012-2013, 46%, 16% and 2% of patients experienced recrudescent infections, respectively, over 63 days of follow-up. Recrudescent parasites had higher prevalence of *K13* mutations (molecular markers of ART resistance), higher PPQ 50% inhibitory concentrations (IC50s), and higher prevalence of Exo-E415G (a molecular marker of PPQ resistance). They also showed lower mefloquine (MQ) IC50s and did not have >1 *pfmdr1* gene (a molecular marker of MQ resistance). We thus hypothesized that these parasites could be effectively treated with artesunate-MQ (AS-MQ) – the former frontline ACT in Cambodia. In 2014-2015, while DHA-PPQ was still the frontline ACT in Cambodia, we measured the efficacy of AS-MQ in 144, 60, and 92 patients in Pursat, Preah Vihear, and Ratanakiri provinces, respectively. In Pursat, Preah Vihear, and Ratanakiri the prevalence of parasites with *K13* mutations increased (77, 34, and 11% to 94, 93, and 18%) and similarly the prevalence of Exo-E415G increased (74, 20, and 3% to 76, 88, and 17%) from 2012-2013 to 2014-2015. Meanwhile, the prevalence of >1 *pfmdr1* gene decreased (8, 14, and 2% to 0, 0, and 0%). Only 1/296 patients presented with a recrudescent infection over 42 days of follow-up, indicating that AS-MQ effectively treats DHA-PPQ-resistant malaria.

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SIGNIFICANT DIFFERENT LEVELS OF ARTEMISININ MONOTHERAPY EFFICACY ON *PLASMODIUM FALCIPARUM* IN MALI

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Resistance to artemisinin derivatives is associated with delayed parasite clearance. In Mali, five years after ACTs were recommended as the first-line treatment for uncomplicated malaria, a prospective artesunate monotherapy efficacy study observed no delayed parasite clearance time. In the context of regular monitoring of artemisinin resistance we repeated the above studies in two sites of Mali. From October 2015 to March 2016, we conducted a prospective study to evaluate the efficacy of artesunate monotherapy in subjects aged 6 months and longer in Bougoula-Hameau and Faladje. Patients with uncomplicated malaria were treated with artesunate for 7 days and followed for 28 days. Parasitaemia was evaluated every 8 hours until three consecutive slides were negatives. MSP2, Ca1 and TA99 polymorphisms was assessed by PCR to distinguish new infections from recrudescent infections. PCT and parasite clearance half-life were calculated using the online WWARN parasite clearance estimator software (PCE).Results were compared with the studies conducted in Bougoula-Hameau in 2011.We included 100 patients in Bougoula-Hameau and 120 others in Faladje. Adequate Clinical and parasitological response (ACPR) was 92.0% and 79.2% in Bougoula-Hameau and Faladje, respectively. After molecular correction cACPRs was 100% at both sites. By 24 hours after treatment initiation, 28% of participants had cleared parasitemia in Bougoula-Hameau, compared with 2.5% in Faladje (P<0.0001). The median parasite clearance time was 32 hours in Bougoula-Hameau (similar to the Bougoula-Hameau 2011 results) but 40 hours in Faladje (P<0.001). The parasite clearance half-life was 2.0 hours (1.66 to 2.23) in Bougoula-Hameau and 2.8 hours (2.39 to 2.99) in Faladje ($p < 0.001$). Only two participants still had parasites at 48 hours in Faladje and no participant in Bougoula-Hameau. Artesunate monotherapy remains effective on *P. falciparum* in Mali but there are significant differences in the level of susceptibility of parasites from different settings of the country.

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CLINICAL EFFICACY OF ARTEMETHER-LUMEFANTRINE IN RELATION TO DRUG EXPOSURE IN CHILDREN WITH UNCOMPLICATED SEVERE ACUTE MALNUTRITION

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In the Sahel, malaria and malnutrition frequently overlap. Severe acute malnutrition (SAM) affects almost all organs and has been associated with a loss of intestinal villosities reducing intestinal absorption of medicines. However, very limited information is available on the pharmacokinetic properties of antimalarial drugs in children with SAM. We assessed artemether-lumefantrine (AL) clinical efficacy in children with SAM compared to those without SAM, with respect to lumefantrine concentration in their blood. Children under 5 with uncomplicated *P. falciparum* malaria were enrolled between November 2013 and January 2015 in Mali and Niger, one third with uncomplicated SAM and two thirds without SAM. The three-day twice daily AL treatment was directly observed and children were followed for 42 days, with PCR-corrected adequate clinical and parasitological response (ACPR) at day 28 as the primary outcome. Lumefantrine capillary blood concentrations were assessed in a subset of participants at different time points including systematic measurement on day 7. A total of 399 children were enrolled. Children with SAM were younger than their non-SAM counterparts (mean 17 versus 28 months, P<0.0001). PCR-corrected ACPR at day 28 was 100% (95% CI: 96.8%-100%) in SAM versus 98.8% (96.4%-99.7%) in non-SAM, P=0.236. In the age stratified analyses, SAM was associated with a greater risk of reinfection by day 28 in children older than the median of 21 months (hazard ratio=2.25 [1.12-4.48], P=0.022) and day 7 lumefantrine concentrations were significantly lower in SAM (median 251 versus 365 ng/ml in non-SAM, P=0.049). This study shows comparable clinical efficacy of standard doses of AL in children with and without SAM, but a higher risk of reinfection in older children suffering of SAM probably associated with poorer exposure to ACTs, as documented by a lower lumefantrine concentration on day 7. Sub-therapeutic concentration of a drug does not necessarily translate into lower efficacy but could contribute to selecting resistant parasites at population level. Further studies of dose optimization of AL in SAM children are urgently needed.

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ZINC-FINGER NUCLEASE-MEDIATED GENE EDITING ILLUSTRATES THE ROLE OF PFMDR1 N86Y IN MODULATING *PLASMODIUM FALCIPARUM* SUSCEPTIBILITY TO ARTEMISININ-BASED COMBINATION THERAPIES

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Malaria chemotherapy relies heavily on the use of artemisinin-based combination therapies (ACTs), which pair a fast-acting, short half-life artemisinin derivative with a longer half-life partner drug. The current arsenal of partner drugs include lumefantrine, mefloquine, amodiaquine and piperaquine. The recent emergence of resistance to artemisinin has placed increased pressure on these partner drugs, with piperaquine failures increasingly documented in in western Cambodia. Epidemiological studies have suggested that *Plasmodium falciparum* susceptibility to many of these agents can be modulated by mutations at positions 86 or 184 in the digestive vacuole-resident transporter protein PfMDR1. To characterize the role of these PfMDR1 mutations, we employed zinc-finger nuclease-mediated genome editing to modify pfmdr1 in genetic strains harboring Asian/African or South American alleles of the related transporter PfCRT. Our data show a significant role for PfMDR1 N86Y in increasing *P. falciparum* susceptibility to lumefantrine, mefloquine and dihydroartemisinin. This polymorphism also decreased parasite susceptibility to chloroquine and amodiaquine. We also observed a modest, strain-dependent reduction in susceptibility to piperaquine with the PfMDR1 N86/184F haplotype. We also mapped these PfMDR1 variants, as well as copy number changes, globally using 2,500 African and Southeast Asian genomes recently released by the MalariaGEN consortium. These data, combined with findings from our pfmdr1-modifed lines, help inform region-specific drug use policies by predicting changes in parasite susceptibility to current ACTs.

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UNSUPERVISED PRIMAQUINE FOR *PLASMODIUM VIVAX* RADICAL CURE LACKS EFFECTIVENESS IN SOUTHERN PAPUA

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Primaquine is the only licensed drug for eradicating *Plasmodium vivax* hypnozoites and therefore preventing relapses. It is efficacious when prescribed in clinical trials but its effectiveness in real-world settings is unknown. Using individualised hospital surveillance data from southern Papua, Indonesia, we conducted a retrospective cohort study to determine the effectiveness of unsupervised primaquine in combination with dihydroartemisinin-piperaquine for preventing representation to hospital with vivax malaria. Between April 2004 and December 2013 there were 62,492 episodes of vivax malaria available for analysis. The risk of representation with vivax malaria within one year was 34·4% (95% Confidence Interval (95% CI) 33·8-35·0%) after initial vivax monoinfection. Prescription of any dose of primaquine was associated with an Adjusted Hazard Ratio (AHR) for representation with vivax malaria of 0·89 (95% CI 0·85-0·93, p<0.001). Limiting the comparison to high dose (≥5mg/kg total dose) versus no primaquine in the period during and 6 months either side of a large, unplanned primaquine stock outage attenuated substantially this difference (AHR 0·95, 95% CI 0·88-1·02, p=0·15). Unsupervised primaquine for *P. vivax* malaria, prescribed according to international guidelines, had minimal impact on the risk of clinical recurrence within one year. New strategies for effective radical cure are needed urgently.

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ESTIMATING THE RISK OF *PLASMODIUM VIVAX* RELAPSES IN AFGHANISTAN

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Plasmodium vivax is responsible for more than 90% of laboratory-confirmed malaria cases in Afghanistan and continues to cause considerable morbidity in several areas of the country. Control of vivax malaria requires radical cure with primaquine to prevent relapse from

hypnozoite forms, although the proportion of cases with liver forms has not been determined. In Nangarhar and Kunar provinces, Afghanistan, between 2009 and 2014, we undertook an open, randomised controlled trial in patients with vivax malaria, comparing chloroquine plus primaquine (0.25mg/kg/day for 14 days) with chloroquine treatment alone. In patients randomised to the primaquine arm, G6PD deficiency was excluded by the fluorescent spot test prior to treatment. Patients were followed-up for one year; at recurrence, treatment was as at baseline. Preliminary analyses indicate that 588 patients were enrolled (295 chloroquine, 293 chloroquine plus primaquine) with 84.9% completing follow-up or having a *P. vivax* recurrence by 12 months. During the first 6 months, 45 / 263 (17.1%) patients in the chloroquine group had *P. vivax* recurrence, while only 7 / 262 (2.7%) had recurrence in tbe chloroquine plus primaquine group (p<0.0001). In the 6-12 month period, 40 / 205 (19.5%) patients in the chloroquine group had *P. vivax* recurrence, while 30 / 242 (14.0%) had recurrence in the chloroquine plus primaquine group (p = 0.127). This large randomised study shows clearly that there is a signicant store of hypnozoites in patients presenting with *P. vivax* in Afghanistan, and that unsupervised primaquine reduces relapse in the first 6 months by at least 5-fold. G6PD testing will be needed for primaquine deployment at scale. Further analyses will be presented in the meeting.

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IMPACT OF NEW COMBINATION LLINS ON ENTOMOLOGICAL MEASURES OF MALARIA TRANSMISSION IN MALI

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Long-lasting insecticidal nets (LLIN) are a key component to malaria control in sub-Saharan Africa, but widespread pyrethroid resistance may threaten their effectiveness. Combination LLIN containing pyrethroid insecticides plus piperonyl butoxide (PBO) are a possible mitigation strategy, but field evaluations are needed to determine if performance against resistant vectors is superior to standard LLIN. In this study, 16 Malian villages in areas where pyrethroid resistance had previously been documented were randomized to receive one of four types of PBO-containing or traditional LLIN (permethrin+PBO, deltamethrin+PBO, permethrin only, or deltamethrin only). Indoor resting mosquitoes were collected in all villages bimonthly over two years (2014-2015) using pyrethrum spray catch and Prokopack aspirator. The primary outcome measure was the sporozoite rate (proportion of An. gambiae s.l. mosquitoes positive for *P. falciparum* sporozoites); a secondary outcome was indoor resting vector density. Bottle bioassays containing permethrin or delamethrin plus PBO resulted in significantly greater mortality than assays containing only permethrin or deltamethrin ; however, none of the PBO-containing assays restored full insecticide susceptibility (mortality >97%). During high malaria transmission seasons (June, August, October) in both years, sporozoite rates were not significantly different between the permethrin+PBO and permethrin only net arms: 5.1% (95% CI: 4.0, 6.2) versus 5.5% (95% CI: 4.2, 6.7) (p=0.67). Sporozoite rates were significantly lower in the deltamethrin+PBO arm compared to the deltamethrin only arm: 1.9% (95% CI: 1.2, 2.7) versus 3.7% (95% CI: 2.6, 4.8) (p=0.01). Over the 2014-15 rainy season morning indoor resting collections yielded a mean of five and three An. gambiae s.l. per house/night for the deltamethrin+PBO and deltamethrin only net arms, respectively. Overall, there was some evidence that deltamethrin+PBO LLIN were more effective in reducing sporozoite rates in pyrethroid resistant An. gambiae s.l.; however, deltamethrin+PBO nets did not reduce vector density compared to deltamethrin only LLIN.

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IMPACT OF COMBINING INDOOR RESIDUAL SPRAYING AND LONG-LASTING INSECTICIDAL NETS ON *ANOPHELES ARABIENSIS* IN ETHIOPIA: PRELIMINARY FINDINGS OF A RANDOMIZED CONTROLLED TRIAL

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The current malaria vector control interventions, indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) have been used in combination in sub-Saharan Africa with inconclusive evidence that the combined intervention is more effective than either IRS or LLINs alone. In Ethiopia, both interventions target *Anopheles arabiensis*, the sole primary malaria vector. This study compared the impact of combining IRS and LLINs with either intervention alone in south-central Ethiopia. Villages were randomly allocated to four study arms: IRS + LLIN, IRS, LLIN, and control. LLINs (PermaNet 2.0) were provided free of charge. IRS with propoxur was applied before the main malaria transmission season in 2014 and 2015. Adult mosquitoes were collected in randomly selected villages in each arm using CDC light trap catch (LTC) set close to a sleeping person, pyrethrum spray catch (PSC), and artificial pit shelter (PIT), for measuring host-seeking density (HSD), indoor resting density (IRD), and outdoor resting density (ORD). Human landing catch (HLC) was performed in selected villages to monitor An. arabiensis biting behaviors. Mean densities were compared using incidence rate ratio (IRR) calculated by negative binomial regression. A total of 1786 female anophelines of four species was collected of which An. arabiensis (n=574) was highest in the control arm (51.4%) followed by LLIN (31.5%), IRS (9.2%), and IRS+LLIN (7.9%). The mean HSD of An. arabiensis in the IRS+LLIN arm was similar to either the IRS arm (0.03 vs. 0.03/ house/LTC/night) or the LLIN arm (0.03 vs. 0.10/house/LTC/night, p=0.07) and so was the difference in IRD and ORD between the IRS and LLIN compared to the IRS arm. However, both IRD and ORD were higher in LLIN compared to IRS+LLIN (p < 0.001 for indoors). In all study arms, An. arabiensis was actively biting indoors and outdoors throughout the night with an early night biting peak before the local people retire to bed. IRS+LLIN compared to IRS had equal powerful impact on resting density of An. arabiensis, but LLIN had the least impact.

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IMPLEMENTATION OF A NON-PYRETHROID INSECTICIDE-TREATED DURABLE WALL LINING FOR MALARIA CONTROL UNDER OPERATIONAL CONDITIONS IN RURAL TANZANIA

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Despite considerable reductions in malaria achieved by scaling-up long-lasting insecticidal nets (LLINs) and indoor residual spraying, maintaining community protection is challenging. An insecticide treated wall lining (ITWL) has been developed that can be attached to inner house walls and releases a mixture of two non-pyrethroid insecticides over 3-4 years. The operational success of ITWL will be determined not only by its demonstrable efficacy (being evaluated in a 44-cluster randomized trial in Muheza, Tanzania) but also its feasibility, household acceptability and durability under field conditions. To install the ITWL, we recruited 110 teams comprising two local installers and one team leader. Cluster supervisors each supervised 5 teams. Over 6 months in 2015-16, these teams installed ITWL in 5666 rural households (67.7% of all enrolled houses). Completion rate by village varied dramatically (range 42.5%-95.8%). Principal reasons for household refusals included rumors and skepticism, concerns about wall damage, and fear of changing house appearance. Concurrent political elections, higher socio-economic status and skin and eye irritation among 5% of installation workers also lowered initial intervention uptake in some villages. Determining an appropriate scheme for paying installers given differences in house size, providing adequate protective equipment, and reimbursement were a serious challenge. Furthermore, approximately 8% of homeowners consented to installation in only some of eligible rooms, which has potential epidemiological implications. Cross-sectional durability surveys conducted 3 months after installation indicated that ITWLs were no longer installed properly in 33.3% of surveyed rooms, principally due to failed installation fixings (71.8%) and that 91.0% of ITWLs had developed holes from general wear and tear (44.3%) or during actual installation (23.6%). Lessons learned included the need for better communication among supervisors, installers and households, prompt payment of installers, and better protective equipment. Such insights can facilitate future scale up.

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EXPERIMENTAL HUT EVALUATION OF A NEW NON-PYRETHROID INSECTICIDE-TREATED DURABLE WALL LINERS FOR CONTROL OF PYRETHROID RESISTANT *ANOPHELES GAMBIAE* AND *AN. FUNESTUS SENSU STRICTO* IN MUHEZA, TANZANIA

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A new insecticide-treated durable wall liner (ITWL) has been developed which mimics the effect of IRS but is designed to last for 3-4 years. Meant to be used in combination with LLILN, it is made of high-density polypropylene treated with two slow-release non-pyrethroid insecticides. A 9 week experimental hut trial was conducted in Muheza, Tanzania between May-July 2015 in an area with pyrethroid-resistant *Anopheles gambiae* and *An. funestus s.s*. to compare the efficacy of several interventions: including the new ITWL + WHOPES recommended LLIN, ITWL alone, LLIN alone, and pyrethroid wall liner alone. Ceilings were not covered with ITWL. Performance was measured primarily in terms of insecticide-induced mortality. ITWL produced mortality 40-50% of *An. funestus s.s.* and *An. gambiae*. Against *An. funestus s.s*. ITWL alone produced 47% mortality, which was not significantly different to that of LLIN alone (29%, P=0.306) or ITWL + LLIN (35%, P=0.385). Although the numbers of *An. gambiae* were lower, results were similar, with ITWL producing 43% mortality compared with 26% for LLIN. The ceilings provided an untreated refuge for resting mosquitoes. Partial covering of the eaves with DL (1 versus 4 eaves open) had no impact on numbers of either species entering the huts. LLIN provided added personal protection against *An. funestus s.s* over ITWL alone (24% blood-fed for ITWL + LLIN compared with 69% for ITWL only, 1 eave (P=0.001) and 56% (P=0.001) ITWL only, 4 eaves. Cone bioassays of ITWL material after the hut trial produced 98% mortality using pyrethroid-susceptible *An. gambiae* kisumu from the insectary, while F1 offspring of field-collected *An. gambiae* showed lower mortality (80%). Comparison of cone and cylinder bioassays suggested some irritancy from the ITWL. The effect of high community-level coverage of ITWL on epidemiological and entomological parameters of malaria transmission is currently being evaluated in Muheza, Tanzania.

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THE AVECNET TRIAL TO ASSESS WHETHER ADDITION OF PYRIPROXYFEN, AN INSECT JUVENILE HORMONE MIMIC, TO LONG-LASTING INSECTICIDAL MOSQUITO NETS PROVIDES ADDITIONAL PROTECTION AGAINST CLINICAL MALARIA OVER CURRENT BEST PRACTICE IN AN AREA WITH PYRETHROID-RESISTANT VECTORS IN RURAL BURKINA FASO: A CLUSTER-RANDOMIZED CONTROLLED TRIAL USING A STEP-WEDGE DESIGN

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Recent reductions in malaria in sub-Saharan Africa have been associated with increased coverage with long-lasting insecticidal nets (LLINs). Pyrethroids are currently the only insecticide class used for treating nets, and the rapid increase in resistance to pyrethroids in vector mosquitoes may jeopardize future vector control. We assessed whether nets containing a novel combination of permethrin, a pyrethroid, and pyriproxyfen, an insect juvenile hormone mimic, (PPF-LLIN) provide incremental protection against malaria over current best practice of LLINs and prompt treatment in an area with pyrethroid-resistant vectors. In this two-armed step-wedge, cluster-randomized, controlled efficacy trial in Burkina Faso, we used a computerized algorithm to allocated PPF-LLINs randomly to 5 clusters and LLINs to 35 clusters at the start of the trial. One month later, and each subsequent month during the malaria transmission seasons, LLINs were exchanged for PPF-LLIN by groups of 5 clusters, so that 3 months before the end of the 2 year trial all participants had received a PPF-LLIN. A cohort of ~2000 children aged 6 months to 5 years was enrolled by random sampling proportionate to cluster size, and surveyed at the start of the 2014 transmission season and followed in 2014 and 2015 by passive case detection for clinical malaria. Exposure to malaria parasites was assessed by collection of mosquitoes indoors using CDC light traps. The primary endpoints were clinical malaria incidence measured by passive case detection and entomological inoculation rate from *Anopheles gambiae* sensu lato mosquitoes. Generalised Linear Mixed Models will be used to carry out a likelihood ratio test of this primary effectiveness estimate (with alpha=0.05), using Poisson errors, and person-time-at risk as an offset variable. Microscopists reading the slides were blinded. The results from this trial will be presented at the meeting.

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ODOR-BAITED TRAPS AS A NOVEL TOOL FOR MALARIA CONTROL - THE SOLARMAL TRIAL

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Recent progress in malaria control promises that elimination is feasible but to achieve this aim, current tools need to be complemented by novel strategies to combat insecticide and drug resistance. We explored the impact of solar powered, odor-baited mosquito traps on malaria incidence and prevalence on Rusinga Island, Kenya; a location where malaria is endemic and LLINs and drug treatment were already widely available to the population of 25,000. *Anopheles funestus* and An. gambiae s.l. were the main vectors. A system containing a mosquito trap, solar panel, battery, mobile phone charging port and LED lights was termed a SMoTS (solar-powered mosquito trapping system). A novel stepped wedge cluster-randomized control design was used to implement the roll-out of the SMoTS across the island, from zero to complete coverage over 24 months. Three times per year a health and demographic surveillance (HDSS) was conducted covering the entire population while malaria prevalence was measured in a randomly selected 10% of the population. Mosquitoes were monitored over successive six-weekly intervals in 80 randomly selected houses per round. A contemporaneous comparison of clinical malaria incidence was insufficiently powerful to measure an intervention effect due to the unexpectedly low number of clinical cases recorded. Malaria prevalence was significantly lower in intervened compared with non-intervened clusters (29.8% reduction, 95% CI: 20.9 - 38%) and mosquito densities were similarly reduced in intervened clusters, with highest effect on An. funestus (69.2% reduction, 95% CI: 29.1 - 87.4%). It is concluded that the odor-baited traps led to significant reductions in malaria transmission, an effect comparable in size to the impact of LLINs. Sociological investigations showed that the provision of electric light contributed to the high degree of compliance and approval of the SMoTS technology, whereby the population undertook the maintenance of the traps. Mass trapping of mosquitoes should be considered a viable tool for future malaria interventions.

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ANOPHELES DIRUS EFFICIENTLY TRANSMITS MIXED SPECIES AND MULTIPLE CLONE MALARIA INFECTIONS IN CAMBODIA

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Mixed species and multiple clone infections occur commonly in human malaria. It remains unknown whether species and clonal diversity are maintained during transmission in spite of a bottleneck in parasite numbers during Plasmodial transmission to the mosquito. In a study of 12 Cambodians with mixed infections whose blood was membrane fed to reared *Anopheles dirus* mosquitoes, we answered a twofold question. We assessed, first, the relative transmissibility of *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) and second, preservation/loss of Pv/Pf genotypes from patient blood to mosquitoes by deep sequencing at targeted loci. DNA from 30 membrane-fed mosquitoes per patient were subject to real-time species-specific PCR. We found that Pv was transmitted more frequently (7/12) than Pf (2/12) from mixed species infections prior to dihydroartemisinin-piperaquine treatment. Pf was the only species transmitted post-treatment. While few mosquitoes caught in the wild have demonstrated mixed infection, we found that 21% (46/222) of mosquitoes fed on mixed patient blood carried both species. All doubly infected mosquitoes had fed upon blood from patients with concomitant microscopic Pf/Pv gametocytemia. We compared diversity of Pf ama1 and Pvmsp1 haplotypes in patient blood to those at the parasite oocyst and sporozoite stages. Clone diversity (multiplicity of infection roughly 2.6 in both mono and mixed patients) was transmitted to mosquitoes intact, often even on an individual-mosquito basis. In 6/7 patients, all pvmsp1 clones present within infected patients were also found in both the oocyst and sporozoite stages within the mosquito. Assessed individually, mosquitoes often carried multiple clones, with multiplicity of infection similar to that in the blood. Pf infections were mostly monoclonal, but findings were similar. In conclusion we see transmissibility of both Pv and Pf simultaneously from mixed infection patients and the absence of a transmission bottleneck in terms of genetic diversity. The observed transmission efficiency of Pv has important implications for malaria control and models of transmission.

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MESOAMERICAN NEPHROPATHY (MEN) IN NICARAGUA: ACUTE INTERSTITIAL NEPHRITIS OF INFECTIOUS ORIGIN?

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Mesoamerican nephropathy (MeN), a kidney disease of unknown origin, is an unrelenting epidemic, primarily in pacific coastal areas of Central America, resulting in over 20,000 deaths. MeN primarily affects young agricultural workers who lack traditional risk factors for kidney disease, and sugarcane workers in Nicaragua are disproportionately affected. MeN's etiology is a mystery, and no case definition of acute onset has been established. Our goal was to describe the acute clinical scenario of early suspect MeN cases. We conducted a prospective case investigation at a large sugar estate in Nicaragua. Physicians identified patients in the emergency room with decreased kidney function and completed case reports with clinical data and medical history. From Feb 2015-Feb 2016, 255 cases of acute MeN cases were identified, mostly male (90%) and young (median age 29yrs), and glomerular filtration rates (eGFR) were low (mean 48±15 ml/min/1.73m^2). The highest incidence was in June (16% of cases). Frequent symptoms were fever (55%), nausea/vomiting (65%), back pain (58%), headache (47%), and muscle debility (45%). Leukocytosis (75%), with neutrophilia (85%), was characteristic. Almost all had WBC (98%), epithelial cells (94%) and erythrocytes (82%) in urine. Few had history of hypertension (n=8) or diabetes (n=4). Based on this data, we defined an acute case of MeN as: a patient with unexplained impaired kidney function and leukocyturia and 2 or more of the following: (1)nausea/vomiting, (2)back pain, (3)headache, (4)muscle weakness, (5) fever, or (6)leukocytosis or neutrophilia. Our data suggests acute MeN involves systemic inflammation and may reflect acute interstitial nephritis, which can progress to chronic tubulointerstitial nephritis and CKD. Renal biopsy results will confirm diagnosis. We establish an evidence-based case definition of acute MeN to be used to identify new cases in Nicaragua and throughout the region, allowing targeted diagnostics to determine the etiology of MeN.

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SEVERE AND HIGHLY FATAL OUTBREAK OF HISTOPLASMOSIS AMONG TUNNEL WORKERS — DOMINICAN REPUBLIC, 2015

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Histoplasmosis is typically a self-limited illness in immunocompetent persons and can be acquired through inhalation of fungal spores from disturbed bat guano. In September 2015, Dominican Republic (DR) health authorities received reports of several tunnel workers hospitalized with a febrile illness suggestive of histoplasmosis. Outbreaks of histoplasmosis have never been reported in the DR. We investigated to confirm etiology and identify factors associated with severe infection. A case was defined as fever and ≥2 symptoms (headache, constitutional, cough, or respiratory difficulty) in a person who worked in the tunnels during July 30–September 2, 2015. We interviewed workers, reviewed medical charts, and tested serum and urine for *Histoplasma* antigen at the Centers for Disease Control and Prevention (CDC). Thirty-five workers used shovels and wheelbarrows to remove large amounts of bat guano from enclosed, unventilated tunnels without respiratory protection. Thirty (86%) workers had illnesses meeting the case definition, 28 (80%) were hospitalized, 9 (26%) required intensive care unit (ICU) admission, 6 (17%) were intubated, and 3 (9%) died. All were men and none were immunocompromised; median age was 30 (range: 18–62) years. The most common symptoms were fever (83%), cough (77%), and headache (70%). Median time from symptom onset to antifungal treatment was 6.5 days. All intubated patients developed ventilator-associated pneumonia. Eighteen (53%) serum and 13 (42%) urine specimens tested positive for *Histoplasma* antigen. The high case-fatality rate (10-fold higher than typically reported) likely resulted from exposure to a high inoculum of *Histoplasma* in the setting of inadequate respiratory protection, delays in treatment, and high rates of nosocomial infection. Clinicians in the DR should be familiar with the presentation of histoplasmosis to allow for timely recognition and appropriate treatment. The risk of histoplasmosis should be considered in creation and implementation of occupational health and environmental safety standards in the DR.

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PERFORMANCE CHARACTERISTICS OF THE WHATMAN FTA ELUTE CARD AND TAQMAN ARRAY CARD PCR ASSAY AS AN ALTERNATIVE METHOD OF STORAGE OF FECAL SAMPLES AND ENTEROPATHOGEN DETECTION AS PART OF THE TRAVELERS' DIARRHEA TREATMENT TRIAL

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Polymerase Chain Reaction (PCR) assays are increasingly used for pathogen detection in travelers' diarrhea (TD), but their use is limited in field settings due to on-site processing and storage requirements for fecal specimens. The Whatman FTA® Elute Card (FTA card) is an appealing alternative for stool collection, due to its ability to store nucleic acid for prolonged periods at room temperature. We evaluated the performance characteristics of the TaqMan Array Card, a quantitative, singleplex PCR (TaqMan PCR), when performed on diarrheal smears on FTA cards, compared to frozen stool samples. Subjects enrolled in a treatment trial for acute watery diarrhea provided a stool sample prior to treatment, a portion of which was smeared onto a FTA card. FTA cards and frozen stool samples were tested at a central lab, using the TaqMan PCR assay with a broad range of enteropathogen targets. A cycle threshold of 35 was used as the cut-off for positive detection. 153 paired FTA stool cards and frozen stool samples were stored for a median of 23 months (IQR 13-25) before testing. High detection rates were observed for frozen stool (79% [95% CI:73-86%]) and FTA cards (72% [95% CI:65-79%]). The most common pathogens detected in frozen stool were enterotoxigenic *Escherichia coli* (ETEC) (36%), enteroaggregative E coli (EAEC) (36%), enteropathogenic E coli (EPEC) (31%), Norovirus (14%), and Shigella/enteroinvasive E coli (7%). Co-pathogens were detected in 33% of samples, the most common being EAEC and EPEC. Sensitivity and specificity of the TaqMan PCR on FTA cards compared to frozen stool for common enteropathogens was as follows: ETEC (89% [95% CI:78-96%]; 99% [95% CI:94-99%]); EAEC(76% [95% CI:62-86%]; 93%[95% CI:86-97%]); EPEC: (75% [95% CI:60-86%]; 93% [95% CI:86-96%]). No significant decline in performance characteristics was noted with prolonged duration of FTA stool cards. FTA stool cards are a useful alternative to standard collection and storage methods in the field setting, allowing sample storage for several months at room temperature, and exhibiting good performance characteristics when tested with the TaqMan PCR.

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RESULTS FROM THE TRIAL EVALUATING AMBULATORY THERAPY OF TRAVELERS' DIARRHEA (TREAT TD) STUDY: A RANDOMIZED CONTROLLED TRIAL COMPARING THREE SINGLE DOSE ANTIBIOTIC REGIMENS WITH LOPERAMIDE

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A recommended treatment for non-inflammatory travelers' diarrhea (TD) is the combination of an antibiotic, usually a fluoroquinolone or azithromycin, and loperamide. However, adverse events, post-dose nausea with high dose azithromycin, effectiveness of single dose versus multi-dose regimens, limited effectiveness data in Africa, and emerging antibiotic resistance to front line agents, remain a concern impacting evidence-based recommendations. A randomized, double-blind trial was conducted at four sites in Afghanistan, Djibouti, Kenya and Honduras between September 2012 and July 2015. US and UK adults with acute non-inflammatory diarrhea were randomized and received single dose azithromycin (500 mg; 106 persons), levofloxacin (500 mg; 111 persons), and rifaximin (1650 mg, 107 persons) plus loperamide (labelled dosing). Volunteers maintained a symptom diary and were evaluated 1, 3, and 7 days after therapy. The primary efficacy outcome, clinical cure at 24 hours, was evaluated in a non-inferiority trial design (delta, -0.15) with both rifaximin and azithromycin compared to a levofloxacin standard. Time to last unformed stool (TLUS) was a secondary efficacy outcome in addition to safety and tolerability. Clinical cure at 24 hours occurred in 80.2% of the levofloxacin arm, compared to 78.3% and 74.8% in the azithromycin and rifaximin arms, respectively. Compared to levofloxacin, azithromycin was not inferior (p=0.0105). Non-inferiority could not be shown with rifaximin (p=0.033). At 48 and 72 hours, efficacy among regimens was equivalent. Median TLUS among all three arms was no different (azithromycin: 4.0 hours; levofloxacin: 5.6 hours; rifaximin: 5.6 hours). Treatment failures were uncommon (3.8%, 4.5% and 1.9% in the azithromycin, levofloxacin and rifaximin arms, respectively) (p=0.6). There were no differences between treatment groups with respect to post-dose nausea (overall 3.1%, p=0.44), vomiting (overall 1.2%, p=0.7) or other adverse events. Single-dose azithromycin (500 mg), levofloxacin (500 mg) and rifaximin (1650 mg) with loperamide were comparable for treatment of watery diarrhea.

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EFFICACY AND SAFETY OF A SINGLE-DOSE MEBENDAZOLE 500 MG CHEWABLE TABLET IN THE TREATMENT OF *ASCARIS LUMBRICOIDES* AND *TRICHURIS TRICHIURA* INFECTION IN PEDIATRIC PATIENTS: A DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED, PHASE 3 STUDY

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A double-blind, multi-center study was conducted to evaluate efficacy and safety of a single dose of a new chewable, rapidly-disintegrating, mebendazole (MBZ) 500 mg tablet for the treatment of *Ascaris lumbricoides* and *Trichuris trichiura* in pediatric patients (1–16 years). The study had a screening phase (3 days), double-blind treatment phase (DBP, 19 days) and an open-label follow-up (OLP, 7 days). A total of 295 patients, excreting eggs of *A. lumbricoides* and/or *T. trichiura*, were randomized (1:1) (age: mean [SD] =7.8 [3.18] years) and received MBZ or placebo on day 1 in the DBP. All patients were administered MBZ on day 19 (the start of OLP) post repeat stool microscopy analysis. At baseline, 167 patients were infected with A. lumbricoides, 243 with *T. trichiura* and 115 were infected with both. Cure rates (primary efficacy endpoint, end of DBP) were significantly higher in MBZ group vs. placebo group for *A. lumbricoides* (% [95% CI], 83.7% [74.2%; 90.8%] vs. 11.1% [5.2%; 20.1%], p<0.001) and *T. trichiura* (33.9% [25.6%; 42.9%] vs. 7.6% [3.5%; 13.9%], p<0.001). Egg reduction rates (secondary efficacy endpoint) were also significantly higher in the MBZ group vs. placebo for *A. lumbricoides* (97.9% vs. 19.2%; p<0.001) and *T. trichiura* (59.7% vs. 10.5%; p<0.001). There were no deaths or serious treatment-emergent adverse events (TEAEs). Comparable rates and low incidence of TEAEs were reported in the DBP between MBZ (9/144 [6.3%]) and placebo (8/140 [5.7%]) with none of the individual TEAEs being reported in >2 patients in either group. Abdominal pain (n=1), abdominal distension (n=2), and rash (n=1) were the only TEAEs considered possibly related to MBZ treatment. During OLP, the total TEAEs incidence was 2.5% with diarrhea (n=2), abdominal pain (n=1), and vomiting (n=1) considered possibly related to MBZ. All TEAEs resolved spontaneously. In conclusion, a single 500 mg chewable tablet of MBZ was found to have an adequate safety profile and was more efficacious than placebo in the treatment of *A. lumbricoides* and *T. trichiura* in pediatric patients. This new formulation will enable treatment of young children who have difficulty swallowing a tablet.

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ILLNESS AMONG MIGRANTS TO CANADA: SURVEILLANCE REPORT FROM CANTRAVNET SURVEILLANCE DATA, 2015

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Due to ongoing political instability and conflict in many parts of the world, migrants are increasingly seeking asylum and refuge in Canada. We examined demographic and travel correlates of illnesses among migrants to Canada to establish a detailed epidemiologic framework of this population for Canadian practitioners. Data on ill returned Canadian travellers presenting to a CanTravNet site between January 1, 2015 and December 31, 2015 were analyzed. During the study period, 2415 ill travellers and migrants presented to a CanTravNet site, and of those, 519 (21.5%) travelled for the purpose of migration. Sub-Saharan Africa (n=160, 30.8%), southeast Asia (n=84, 16.2%), and south central Asia (n=75, 14.5%) were the most common source regions for migrants, while the top specific source countries, of 98 represented, were the Philippines (n=45, 8.7%), China (n=36, 6.9%), and Vietnam (n=31, 6.0%). Compared to non-migrant travellers, migrants were more likely to have a pre-existing immunocompromising medical condition (p<0.0001) and to require inpatient management of their illness (p<0.0001). Diagnoses such as TB (n=263, 50.7%), viral hepatitis (n=80, 15.4%), nationally notifiable diseases (n=380, 73.2%), and HIV (n=11, 2.1%) were greatly over-represented in the migrant population compared to non-migrant travellers (p<0.0001). Among cases of TB in the migrant population, 82% (n=216) were latent, and 18% (n=47) were active. Compared to non-migrant travellers, migrants were more likely to present with a serious, communicable infectious disease, potentially complicated by an underlying immunosuppressing condition. These differences highlight the divergent health care needs in the migrant population, and underscore the importance of surveillance programs to understand their burden of illness.

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MATERNAL PARASITIC INFECTIONS ALTER INFANT ANTIBODY RESPONSE TO PNEUMOCOCCAL IMMUNIZATION

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Vaccine-preventable diseases remain a significant cause of early childhood mortality in developing countries, despite concerted efforts to improve vaccine coverage. One reason for this discrepancy may be the impact of prenatal exposure to parasitic antigens on the infant's developing immune system. Our goal in this study was to investigate the effect of maternal parasitic infections on the infant immune response to early childhood vaccines. 580 pregnant Kenyan mothers were enrolled in the study and tested at prenatal visits for malaria, soil transmitted helminths, *Giardia lamblia*, *Strongyloides stercoralis* and *Schistosoma haematobium* infection. The infants received the 10-valent *Streptococcus pneumonia* conjugate vaccine (PCV), *Haemophilus influenzae* type B (Hib) and *Diphtheria* toxoid (DT) vaccines at 6, 10, and 14 weeks of age. Serum was collected from cord blood, 10 and 14-weeks, 6 months, and every 6 months following through the second year of life. A multiplex fluorescent bead assay determined IgG concentrations to HiB, DT, and the ten PCV serotypes: 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F. Total parasitic infection incidence among mothers in the study is high, with 373 of 580 (64.2%) participants having at least one parasitic infection during pregnancy and 75 participants (13%) with 2 or more infections. The most common infections were hookworm (19.7%), *Plasmodium falciparum* (16.2%), *S. haematobium* (11%), and *Trichuris trichiura* (10.5%). In preliminary analysis using a mixed linear model comparing infection status to log (antibody concentration), we are able to see a 37% higher concentration of PCV 9V antibody in children born to mothers with infections (p=0.016) compared to uninfected mother/child pairs. *S. haematobium* infection was associated with significantly higher concentrations in 4 PCV antigens: 1 (3.9 fold higher, p=0.0006), 7 (2.3 fold higher, p=0.004), 9V (2.3 fold higher, p=0.002), and 18C (6.8 fold higher, p<0.0001). More testing is ongoing with this study, including more samples from later time-points, which will provide a more detailed analysis of the dynamics of first and second year responses to vaccine antigens.

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POPULATION GENOMICS OF *WUCHERERIA BANCROFTI* FROM ARCHIVED SAMPLES USING SELECTIVE WHOLE GENOME AMPLIFICATION

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Lymphatic filariasis (LF) is a major threat to human health in the tropics, leading to the disfiguring and debilitating conditions hydroceles and elephantiasis. There are approximately 1.2 billion people living in LF endemic countries where 120 million people currently suffer from the disease. The parasites that cause LF are separated into two genera, *Brugia* and *Wuchereria*, with *W. bancrofti* (Wb) responsible for ~90% of LF cases. Until recently, Wb has been neglected in genomic studies due to complications in sample collection and preparation, such as lack of adult stage samples. Our previous work has shown it is possible to concentrate Wb microfilaria from an infected blood sample and produce high quality genomic sequence. Here we expand upon our previous studies in Papua New Guinea to include three more endemic areas of Wb infectivity: Mali, Kenya, and Haiti. We utilize a new method of whole-genome amplification to generate whole-genome sequence data from archived DNA, blood, and PBMC samples containing microfilaria of Wb. Using this method, we have generated whole-genome data for 42 single microfilaria isolates from Haiti (9), Mali (11), Kenya (10), and PNG (12). We report on thousands of unique single nucleotide polymorphisms (SNPs) and identify genes that are highly variable among localities. We utilize discovered SNPs to: i) elucidate the historical context of admixture between endemic areas, retracing the possible routes of Wb migration, ii) root the gene trees of Wb to identify the species origin, and iii) reconstruct the historical demography by whole-genome coalescent models. Our results provide a new context for studying Wb biology and may implicate biological complexities that will hinder elimination. Our methods advance the study of Wb genomics by allowing the sequencing of archived Wb samples. This new data source, when paired with WGS from recent times and epidemiological data, provides a post-hoc method of hypothesis generation. These hypotheses can then be used to predict how different regions infected with Wb may respond to ongoing elimination pressure.

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POST-GENOMIC EMPIRIC IMMUNOMIC ANALYSES IDENTIFY NOVEL BIOMARKERS FOR ACTIVE *ONCHOCERCA VOLVULUS* INFECTION

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Onchocerciasis is a neglected tropical disease that has been targeted for control and elimination through mass drug administration of ivermectin. Currently, surveillance tools have relied on xenomonitoring and Ov16-based IgG4 antibody tests, the latter having sensitivities ranging from 70-80% across most endemic countries. As program goals have shifted from disease control to elimination of *Onchocerca volvulus* (Ov), additional tools may be needed that can be used in both surveillance and in the certification of elimination of onchocerciasis. Empiric analyses of the stage-specific Ov transcriptomes resulted in the identification of 398 proteins that favor exposure and propensity for eliciting antibody responses. These proteins were expressed, gridded on protein arrays and screened for isotype-specific (IgG1, IgG3, IgG4, IgE) responses using 52 individual sera from Ov-infected and appropriate control individuals. Multivariate analyses of isotype reactivity level and infection status resulted in the identification of 15 'Ov-specific' proteins that had significantly higher IgG4 responses ($p < 0.0001$, ANOVA) in infected individuals compared to control sera. The top 5 candidate proteins (OVOC10469, OVOC3261, OVOC10602, OVOC5127 and OVOC11950) were each expressed in mammalian cells as fusion proteins and tested in a luciferase immunoprecipitation system that allowed for the rapid identification of *O. volvulus* - (but not related filarial parasite-) specific targets of IgG4 reactivity. Among these 5 potential biomarkers 3 showed sensitivities ranging between 80-90%, with specificities that approached 100% when tested with a large panel (n=400) of well-characterized sera. When coupled to the IgG4 responses to Ov16, the IgG4 responses to any one of these 3 biomarkers achieved close to 100% sensitivity with no apparent loss of the 100% specificity seen individually. Additional optimization is being performed to configure the next generation of point of care immunoassays. These data provide an important and practical example of how "immunomic" analyses in the post-genomic era can rapidly provide solutions to very practical problems.

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NOVEL BIOMARKERS FOR THE IMMUNE-BASED QUANTIFICATION OF *LOA LOA* MICROFILARIAE AND THE DIAGNOSIS OF LOIASIS

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Some individuals infected with high levels of *Loa loa* (Ll) microfilariae (mf) are known to experience fatal post-ivermectin severe adverse events that have led to the interruption of ivermectin-based mass drug administration programs in some regions of Central and West Africa. Thus, tools that can accurately identify "at risk" individuals (with high Ll mf loads) at the point of contact (POC) are of critical need. To identify potential biomarkers that could be used as the basis of immunologically-based POC assays, we first conducted transcriptomic analysis of the Ll mf and used these data to provide a framework for identifying proteins (through LC-MS/MS analysis) present in the secretory/excretory (E/S) products of Ll mf from *in vitro* cultures. In addition, proteomic analyses of individual plasma (n= 10) and urine (n=6) of Ll-infected individuals compared to uninfected controls were also performed. Of the 12,200 transcripts expressed by Ll mf, 1,166 proteins were found in the Ll mf E/S products. Some of the mf E/S proteins were specifically detected in the urine (n=205) or the plasma (n=25) of Ll-infected individuals. Through a bioinformatics-filtering pipeline, we identified 28 potential Ll mf-specific biomarkers. Among these 28, 2 mf-specific antigens (LOAG_14221 and LOAG_15846) were detected in plasma of Ll-infected patients with little to no reactivity to sera from patients infected with *W. bancrofti* or *O. volvulus*. Interestingly, levels of LOAG_14221 in mf-positive Ll infected patients were positively correlated to measured mf densities in the corresponding blood ($r = 0.6$ and $P = 0.0007$), and LOAG_14221-based assay showed a sensitivity of 76% and a specificity of 78% compared to gold-standard microscopy. Thus, we are in the process of creating additional reagents to configure a quantitative POC rapid immunoassay for LOAG_14221 antigen in plasma and urine as a surrogate for Ll mf quantification using standard techniques.

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ALLERGIC SENSITIZATION UNDERLIES HYPER-REACTIVE ANTIGEN-SPECIFIC CD4+ T-CELL RESPONSES IN COINCIDENT FILARIAL INFECTION

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Among the various hypotheses put forward to explain the modulatory influence of helminth infection on allergic effector responses in humans, the IL-10 induced suppression of Th2-associated responses has been the leading candidate. To explore this helminth/allergy interaction more fully, parasite- and allergen--specific T cell responses CD4+ T cell responses in 12 subjects with filarial infections and coincident allergic sensitization (Fil+A+) were compared these to the responses to 3 appropriate control groups [Fil-A- (n=13), Fil-A+ (n=12), Fil+A- (n=11)]. The most important findings revealed that FIL+A+ had marked ($p<0001$ for all cytokines) increases in parasite antigen-driven Th2 (IL-4, IL-5, IL-13), Th9 (IL-9) and the regulatory (IL-10) cytokines when compared with Fil+A-. Moreover, using multiparameter flow cytometry, filarial parasite antigen induced a marked increase in not only the frequency of CD4+ T cells producing IL-4, IL-5, IL-2 and TNF-α in Fil+A+ when compared to Fil+A- patients but also in the frequencies of polyfunctional Th2-like (CD4+IL-4+IL-5+ and CD4+IL-2+IL-4+IL-5+TNF-α+) cells. The Th2-associated responses seen in the Fil+A+ group was correlated with serum IgE levels ($p<0.01$, $r=0.5165$ for IL-4; $p<0.001$, $r=0.5544$ for IL-5; and, $p<0.001$, $r=0.4901$ for IL-13), levels of circulating eosinophils ($p<0.0116$, $r=0.5656$) and their degranulation/activation products [major basic protein ($p<0.001$, $r=0.7353$) and, eosinophil derived neurotoxin ($p<0.01$, $r=0.7059$)]. CD4+ responses to allergen were not different (to a large extent) among the groups. Taken together, our data suggest that filarial infection drives an augmented parasite antigen-specific T cell response characterized by a Th2-dominated immune response that largely is pro-allergenic. This response, while possibly able to limit parasite burden, may be responsible for the induction of parasite-associated (but pro-allergic) pathology.

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SCHISTOSOMA MANSONI INFECTION IMPAIRS REPRODUCTION IN MICE

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Schistosomiasis is a neglected tropical disease, endemic in 76 countries, that afflicts more than 240 million people. The impact of schistosomiasis on infertility may be underestimated according to recent literature. Extracts of *Schistosoma haematobium* include estrogen-like metabolites termed catechol-estrogens that down regulate estrogen receptors alpha and beta

in estrogen responsive cells. In addition, schistosome derived catechol-estrogens induce genotoxicity that result in estrogen-DNA adducts and cause hormonal imbalance. We now hypothesize the induction of infertility in individuals infected with *S. mansoni* also through an hormonal imbalance. The aim of this study was to study infertility in mice infected with *S. mansoni*. Female mice infected with *S. mansoni* and noninfected female controls, male mice infected with *S. mansoni* and noninfected male controls were mated during 8 months. Gestational length and, number of pupps were studied. Animals were euthanized and their ovaries, uterus and testes were examined histopathologically. Infected females had shorted gestational length than controls. Births of infected females were not synchronous as in controls. The number of pupps was decreased in infected females in comparison to controls. Ovaries, uterus and testes of infected mice showed definite structural damage. No ova, worms or specific granulomata were detected in infected mice in organs other than liver and spleen. To our knowledge this is the first study addressing *S. mansoni* infection associated infertility. It is concluded that schistosomiasis has an important metabolic effect leading to reproduction disorder in infected animals. These results together with histopathological findings with absence of egg in all examined ovaries, uterus and testicular sections emphasize the possible role of hormonal imbalance in the pathogenesis of such lesions. The changes observed could be due to catechol-estrogens associated with schistosomiasis.

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THE EFFECTS OF PRAZIQUANTEL ON THE TEMPORAL INTERACTION BETWEEN THE HELMINTH PARASITE *SCHISTOSOMA MANSONI* AND ITS MURINE HOST

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After penetrating the skin of its mammalian host, *Schistosoma mansoni* schistosomula migrate via the bloodstream to the liver and mesenteric veins where males and females mature and pair to produce fertile eggs. While many of these eggs are excreted in the feces, a number become lodged in the liver where they drive granuloma formation that causes much of the pathology associated with the disease. Praziquantel (PZQ) is the only drug available for treatment of schistosomiasis. Although the drug kills sexually mature worms it is unable to cure infection because it is ineffective against sexually immature juvenile worms 2-4 weeks after infection. The molecular basis of juvenile resistance to PZQ remains unknown and here we investigate the potential role of ATP Binding Cassette (ABC) transporter genes in the differential response of adult and juvenile *S. mansoni* to PZQ treatment. Expression data for 16 *S. mansoni* genes including 9 ABC transporters over the treatment period will be shown. Our data suggests there is significant differential expression of transporters between *S. mansoni* adult versus juvenile in response to PZQ. In addition, we have employed next generation RNA sequencing technology (Illumina) together with the Lumenogix data analysis platform to examine the differential expression of mouse hepatic genes during *S. mansoni* infection. *S. mansoni* infected mice were treated with a lethal dose of PZQ over 4 consecutive days beginning on either day 25 (juvenile *S. mansoni*) or day 32 (adult *S. mansoni*) post infection. Infected liver tissue was excised and total RNA extracted. The differential expression of immune, fibrotic, and inflammatory genes and pathways will be reported and correlated with egg deposition and granuloma formation in the murine liver.

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DENGUE VIRUS: THE CIRCULATION OF FOUR SEROTYPES IN AN ENDEMIC REGION, DURING NINE SEASONS: SINGULARITIES ON EPIDEMIC DYNAMICS AND GENETIC DIVERSITY

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Dengue virus (DENV) is a Flavivirus of great importance in public health, especially in tropical regions that present favorable environment for mosquito vector development. This study presents a molecular surveillance of dengue occurred in São José do Rio Preto, São Paulo State, Brazil, during nine epidemiological seasons (August 2005 to July 2014). Patients with typical symptoms of dengue who were attended in the public health system had blood samples collected for DENV detection. A total of 1,774 samples were DENV positive. They were identified 511 (27.80%) DENV1, 202 (11.38%) DENV2, 494 (27.84%) DENV3 and 567 (32.96%) DENV4. DENV serotypes circulation can be described as it follows: DENV1 was mainly detected from 2009 to 2012; DENV2 was detected from 2008 to 2012; DENV3 was identified in 2006 but no longer detected after 2007 and finally, DENV4 was detected 2011 onwards. The four dengue serotypes have been detected, representing a hyperendemicity scenario. Phylogenetic reconstructions of 4 serotypes were conducts from 81 complete genome 74 complete sequences of the envelope gene (E Protein). The genotypes circulating were: DENV1 genotype V, DENV2 Asian/American genotype, DENV3 genotype III and DENV4 genotype II. DENV1 strains are from two different lineages, with specific amino acids for each lineage and two dates of introduction: 2008 up to 2014 (MRCA estimated to 2006) and 2010 up to 2012 (MRCA estimated to have existed in 2008). Two subgroups of DENV2 were founded, with specific amino acids changes for one: First group that include 2008 strains (MRCA dating probably in 2004) and other group with strains from 2006, 2011 up to 2014 (MRCA dating probably in 2006. DENV 3 and DENV4 showed one lineage each one and the serotypes circulating are the same as described previously, on Brazil. The co-circulation of multiple serotypes resulted in competition, and genetic diversity. Dengue surveillance is important to understand the mechanisms of introduction and extinction of strains and replacement of serotypes, genotypes or lineages.

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EVALUATION OF THE HEALTH-RELATED QUALITY OF LIFE OF CHILDREN WITH DENGUE AND MALARIA IN WESTERN KENYA

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Malaria and dengue are leading causes of mortality and morbidity in sub-Saharan Africa. Although acute disease symptoms can be clinically recognized, the effects on daily living associated with disease due to either or both infections are still unclear, especially in a pediatric population where disease burden is the greatest. The goal of this study was to investigate the effect of dengue virus (DENV) and/or malaria infection on health-related quality of life (HrQoL) in children in Western Kenya. We used the Pediatric Quality of Life Inventory (PedsQL), a modular

instrument, to assess health-related quality of life (HrQoL) among children, aged 2-18 (n = 171), who presented with fever to one of two health centers in Western Kenya. The PedsQL questionnaires were administered to subjects and their parents in their native Luo language. Blood samples from child with acute febrile illness were assayed for malaria parasitemia by light microscopy or by rapid diagnostic testing cards, and for DENV viremia by RT-PCR. Cases of DENV infection were also identified based on anti-DENV IgG seroconversion by ELISA between blood samples obtained at time of presentation and at one month follow up. The mean PedsQL score for all febrile children was 87.3 (95% CI 85.6 to 89.1) at the time of presentation. By the one month follow up visit, the mean score increased to 94.9 (95% CI 93.5 to 96.4, p<0.001 by paired T-test). The increases by the convalescent visit were also observed for groups of children who had malaria (mean score 88.5 to 96.1, p<0.001) or who had febrile illness that was neither malaria nor DENV (mean score 87.7 to 96.3, p<0.001). For children who were infected with DENV, or who had concurrent infection with both DENV and malaria, the increase in scores was less pronounced; the mean score increased from 84.7 to 91.1 (p=0.3) and 83.6 to 90.7 (p=0.07) for children with DENV mono-infection or DENV/malaria co-infection, respectively. Data are continuing to be collected as part of our ongoing study. However slower recovery in the PedsQL scores of DENV-infected children may indicate that consequences of DENV infection may have longer-lasting effects than previously believed.

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MOLECULAR CHARACTERIZATION OF TWO MAJOR DENGUE OUTBREAKS IN COSTA RICA

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Dengue virus (Flavivirus; Flaviviridae) is a re-emerging arthropod-borne virus with worldwide circulation transmitted mainly by *Aedes aegypti* and *Ae. albopictus* mosquitoes. Since the first detection of its main transmitting vector in 1992 and the invasion of DENV-1 in 1993, Costa Rica has faced dengue outbreaks yearly. In 2007 and 2013 Costa Rica experienced two of the largest outbreaks in terms of total and severe cases. In order to provide genetic information about the etiologic agents producing these outbreaks we conducted phylogenetic analysis of viruses isolated from human samples. A total of 23 DENV-1 and DENV-2 sequences were characterized. These analyses signaled that DENV-1 genotype V and DENV-2 American/Asian genotype were circulating in those outbreaks. Our results suggest that the 2007 and 2013 outbreak viral strains of DENV-1 and DENV-2 originated from nearby countries and underwent in situ microevolution.

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ARTHROPOD EXOSOMES AS NOVEL TRANSMISSION BLOCKING STRATEGIES FOR VECTOR-BORNE PATHOGENS

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Molecular determinants and mechanisms of arthropod-borne flavivirus transmission to the vertebrate host are poorly understood. The transmission strategies used by flaviviruses to exit arthropods and infect human host were envisioned as best approaches to develop transmission-blocking vaccines against molecules or determinants that facilitate pathogen transmission. Research in my laboratory has shown that both tick and mosquito-borne flaviviruses uses exosomes, the small membranous extracellular vesicles for transmission from arthropods to human host. Our studies have revealed that arthropod derived exosomes are important means of communication and transmission between the vector and the vertebrate host. We have found that Langat virus (LGTV), a flavivirus member closely related to tick-borne encephalitis virus and mosquito-borne dengue viruses are transmitted from vector to the vertebrate host through exosomes. The exosomes containing LGTV and dengue viruses were viable, secured and highly virulent in all tests such as re-infection kinetics, trans-migration, inhibitor studies and viral plaque formation assays suggesting exosomes as favorable modes of transmission. Both matured virions and replicative forms of arthropod-borne flaviviruses were found to be using exosomes for transmission. Our data also showed that arthropod derived exosomes facilitated infection of human cells that eventually produced exosomes loaded with flaviviruses. Our current efforts are focused on understanding the molecular mechanisms/associated signaling cascades and virus conformations in exosomes derived from arthropods and arthropod cells. Overall, our studies would suggest the arthropod derived exosomes as novel transmission blocking strategies for treating flavivirus infections.

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PLASMODIUM FALCIPARUM CO-INFECTION MODULATES DENGUE DISEASE SEVERITY

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Severe dengue virus (DENV) disease is an important cause of childhood mortality worldwide. Due to lack of surveillance, the burden of DENV infection in Sub-Saharan Africa is unknown, but likely underestimated. Further, since the DENV mosquito vector shares a geographic distribution with that of the *Plasmodium falciparum* (*Pf*) vector, concurrent infection with both pathogens can occur. Although DENV/malaria co-infection is increasingly recognized in other parts of the world, reports of DENV/*Pf* co-infection are sparse in Africa due to under-recognition of DENV disease. Consequently, little is known about the disease manifestations of DENV/*Pf* co-infection, particularly in children, who are known to bear a larger burden of both diseases. The purpose of the present study is to bridge gaps in our understanding of DENV/*Pf* co-infection disease. As part of our ongoing study on arboviral infection in Kenyan children, we enrolled children who presented with fever of unclear etiology to one of four centers located in Kisumu County in western Kenya, and Kwale County on the Kenyan coast. To date, 579 blood samples from febrile children (mean age 4.3-years) have been tested for both DENV RNA by RT-PCR and malaria by light microscopy. 333 (58%) were positive for *Pf*. 73 (13%) samples were positive for DENV. 33 (49%) of the DENV-positive samples also were positive for *Pf*. Children with DENV/*Pf* co-infection or *Pf* mono-infection were older than DENV mono-infected children (mean age 5.1 and 4.8 years vs. 3.4 years, respectively, p=0.008). DENV/*Pf* co-infected and *Pf* mono-infected children also had higher fevers at presentation than did DENV mono-infected children (mean temperatures of 39.0 and 38.8 vs. 38.5 degrees C, respectively, p<0.01). Compared with *Pf* mono-infected children, body aches, joint tenderness, and splenomegaly were observed more frequently in DENV mono-infected children, but less frequently in DENV/*Pf* co-infected children. These preliminary findings suggest that concurrent infection with *Pf* may exert a modulatory effect on clinical DENV disease. Further investigation of DENV/*Pf* co-infection will yield insights important for clinical care.

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LOWER T CELL APOPTOSIS IN THE SECOND INFECTION WITH HETERO-SEROTYPE DENV

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The available evidence suggests that dengue virus-specific T lymphocytes and cytokine storm play a pivotal role in the immunopathogenesis of plasma leakage. Investigations are underway to identify the immune profiles associated with increased or decreased risk for severe disease. In this study, CD14+ cells from the peripheral blood mononuclear cells (PBMCs) of patients who recovered from DENV-1 infection were infected with DENV-1 or DENV-2 and co-cultured with memory T cells. We found that secondary infection with DENV-2 suppresses the cell reproductive capacity but forms more cell clones and more functional cells to produce more proinflammatory factors (IFN-γ, TNF-α, IL-6, IL-8, IL-12 and IL-17) and less regulatory cytokines (IL-10, TGF-β) which results in higher viral replication compared to secondary infection with DENV-1. Memory dengue virus-specific T cells which are induced in a primary dengue virus infection are reactivated by the heterologous serotype of dengue virus and antigen-presenting cells (APCs) during a secondary infection. Dramatically, less apoptosis and more continuous activation of T cells in secondary infection with hetero-serotype DENV were observed. This discovery which has not been reported previously may be the reasonable and vital interpretation for the cytokine storm and severe symptoms observed in secondary infection with DENV. In summary, secondary infection with hetero-serotype DENV elicits the relatively pathological immune response while secondary infection with homologous-serotype DENV induces the relatively protective immune response by activation-induced cell death (AICD) of T cells.

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PREDISPOSING SECOND IN ADULT DENGUE PATIENTS

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Dengue, a major public health problem in the world, is now endemic in more than 100 countries. It is estimated to have 390 million Dengue infections with 96 million cases worldwide. Mortality rate can be high ranging from 0.1% to 5%. Bleeding is a common complication in Dengue which can even lead to death if not detected and treated early. Therefore, identifying predictors of bleeding would be very valuable as such patients could be closely monitored to detect and treat bleeding early. A prospective Case Control Study was conducted to determine the incidence of bleeding, type of bleeding and possible predictors of bleeding. All patients admitted to Dengue Management Unit at the National Institute of Infectious Diseases, Colombo, Sri Lanka for a period of four months from 1st of July 2014 were included in the study. Dengue infection was confirmed by NS1 antigen or Dengue Specific IgM antibodies. These patients were followed up to see the development of bleeding, possible effects of bleeding and the need of blood transfusion.There were 1000 patients with confirmed Dengue infection with 546 males and 454 females. Age ranged from 12 to 86 years. (mean 31 yrs.) 56.2% (n=562) had DF; 43.8% (n= 438) had DHF. 332(33.2%) had some degree of bleeding; major bleeding in 17.0%, minor bleeding in 15.9%; 67.1% had no bleeding at all, other than petechial bleeding. 81(8.1%) needed therapeutic blood transfusions. Major bleeding was significantly more common ($p<0.05$) in those who had severe vomiting, postural dizziness, abdominal pain and hepatic tenderness and those who had NSAIDS. Females had more bleeding than men ($p<0.05$). Obese patients had a higher risk of having bleeding ($p<0.05$), but not overweight patients(BMI 23-27). Patients with platelet counts less than 50,000 per cmm were at a higher risk of having bleeding as well as DHF patients. This study identifies predictors which would put Dengue patients at high risk of bleeding. This will enable clinicians to monitor such patients carefully to detect and to treat bleeding promptly thereby reducing morbidity and mortality.

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PREVALENCE OF DENGUE AND CHIKUNGUNYA VIRUS INFECTIONS IN NORTHEASTERN TANZANIA: A CROSS SECTIONAL STUDY AMONG PARTICIPANTS PRESENTING WITH MALARIA-LIKE SYMPTOMS

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Abstract In spite of increasing reports of dengue and chikungunya activity in Tanzania, limited research has been done to document the general epidemiology of dengue and chikungunya in the country. This study aimed at determining the sero-prevalence and prevalence of acute infections of dengue and chikungunya virus among participants presenting with malaria-like symptoms in three communities with distinct ecologies of north-eastern Tanzania. Cross sectional studies were conducted among 1100 participants (aged 2-70 years) presenting with malaria-like symptoms at health facilities at Bondo dispensary (Bondo, Tanga), Hai hospital (Hai, Kilimanjaro) and TPC hospital (Lower Moshi). Participants who were malaria negative using rapid diagnostic tests (mRDT) were screened for sero-positivity towards dengue and chikungunya Immunoglobulin G and M using ELISA-based kits. Participants with specific symptoms defined as probable dengue and/or chikungunya by WHO were further screened for acute dengue and chikungunya infections by PCR. Out of a total of 1100 participants recruited, 91.2% (n=1003) were malaria negative by mRDT. Out of these, few of the participants (<5%) were dengue IgM or IgG positive. A total of 381 participants had fever out of which 7.9% (30/381) met the defined criteria for probable dengue, though none (0%) was confirmed to be acute cases. Chikungunya IgM positives among febrile participants were 12.9% (49/381) while IgG positives were at 3.7% (14/381). A total of 69.0% (263/381) participants met the defined criteria for probable chikungunya and 4.2% (11/263) were confirmed by PCR to be acute chikungunya cases. Further analyses revealed that headache and joint pain were significantly associated with chikungunya IgM seropositivity. In north-eastern Tanzania, mainly chikungunya virus appears to be actively circulating in the population. Continuous surveillance is needed to determine the contribution of viral infections of fever cases. A possible establishment of arboviral vector preventive control measures and better diagnosis of pathogens to avoid over-treatment of other diseases should be considered.

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NATURAL ANTIBODY RESPONSES TO THE CAPSID PROTEIN OF DENGUE

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The capsid (C) protein is a structural protein of the dengue virus (DENV) that encloses its genetic material. Few previous studies reported antibody responses to DENV C protein during natural infection. To further understand this, we studied natural antibody responses against C protein of all four DENV serotypes. Antibody responses of sera obtained from dengue seropositive healthy volunteers (DENV1 n=8: DENV2 n=8: DENV3 n=5 and DENV4 n=8) from Sri Lanka, were screened against an array of 14 peptides, representing the entire C protein sequence of DENV2, using

ELISA assay. The peptides are of 15- to 18-amino acids (aa) in length, with 10 aa overlaps. Three peptides P1 (2-18 aa), P11 (79-95 aa) and P12 (86-101 aa) showed positive responses (cut-off = mean OD of 8 negative controls + 3 standard deviation) to sera from individuals infected with all four DENV serotypes. For P11 and P12, 100% of sera from each serotype were positive, whereas for P1, 100% sera from DENV1, 3 & 4 and 86% sera from DENV2 were positive. These peptides are located on N and C terminal regions (1-40 and 70-100 respectively) which are characterized to be highly hydrophilic, surface accessible and flexible regions. According to IEDB conservancy analysis, the overall conservancy % values across the four serotypes of the three positive peptides: P1, P11 and P12, are 44%, 64% and 58% respectively. Two peptides P6 (39-56 aa) and P10 (71-89 aa) were positive only against the sera of individuals who had been infected with DENV2 and DENV4 (100% positive). Remaining nine peptides P2 (8-25 aa), P3 (16-32 aa), P4 (23-40 aa), P5 (40-48 aa), P7 (47-63 aa), P8 (54-71 aa), P9 (62-79 aa), P13 (93-110 aa) and P14 (100-114 aa) did not show significant positive antibody responses. Out of those: P5, P7 and P8 are located in a hydrophobic region and therefore not likely to be potentially antigenic. These results further provide evidences for DENV C protein being immunogenic in natural infections.

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COCULTURE OF ENDOTHELIAL CELLS AND MONOCYTES AS A POTENTIAL MODEL TO STUDY DENGUE PATHOGENESIS AND SCREEN COMPOUNDS WITH THERAPEUTIC POTENTIAL

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Plasma leakage is one of the main signs of complication of dengue virus (DENV) infections and it is related to the disease severity. The lack of animal models representing satisfactorily the pathophysiology of dengue fever in humans has been limiting the advances in understanding the disease mechanisms as well as the development of drugs and vaccines for dengue management. Many studies have been shown that monocytes are one of the main cells responsible for immune response to DENV, producing mediators that interact with endothelia increasing vascular leakage in humans. Therefore, the aim of this work was to stablish a model for assessing the *in vitro* vascular permeability using endothelial cells co-cultured with monocytes infected by DENV. Monocytes (THP-1 cells) were infected with DENV-2 strain ACS 46, at MOI of 0.1 and 1, and put in contact with endothelial cells (HUVEC) monolayers through apical or basolateral side of transwell inserts. As controls, monocultures of HUVECs were infected likewise. UV inactivated virus and supernatant of mock infections were also tested. The endothelial barrier function was evaluated by measuring the Transendothelial electrical resistance (TEER). Data were compared by one-way ANOVA/Tukey's test with $p<0.05$. Results show that the coculture system, infected at MOI of 0.1 , presented significant lower TEER values in comparison to the infected monocultures of endothelial cells, as well as the cocultures infected with UV inactivated virus or supernatant of mock infections, after 24 h of basolateral contact. No differences were observed at the same conditions with apical contact. At MOI of 1, we found no difference between mono- or cocultures. These results indicate that the infected coculture more efficiently interfered with the endothelial barrier function *in vitro* at low MOI (0.1), being a potential mimicry model of plasma leakage triggered by dengue infections. The proposed system will allow us to screen of compounds with therapeutic potential and study the interference of different immunomediators on vascular permeability in order to better understand the pathogenic factors associated with severe outcomes.

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EFFICACY OF RUPATADINE IN THE TREATMENT OF ACUTE DENGUE INFECTION

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Our previous studies showed that platelet activating factor (PAF) was a potent mediator of vascular leak. Therefore, we proceeded to investigate the efficacy of rupatadine which is a PAF receptor blocker in patients with acute dengue infection. We conducted a phase II, open label, randomized placebo controlled trial to determine the safety of rupatadine in patients with acute dengue, the efficacy of rupatadine in preventing or reducing vascular leak and to determine its efficacy in reducing complications associated with acute dengue. The study was carried out in 3 arms: rupatadine 40mg daily, rupatadine 10mg daily and the placebo. The patients were examined and laboratory parameters were measured at least twice a day to detect any complications and fluid leakage. Daily ultrasound scans were done from the day of admission to determine the presence and the quantity of fluid leakage. 138 patients were recruited on day 4.8 of illness (SD±0.55) with 44 receiving 40mg daily rupatadine, 44 receiving 10mg daily rupatadine and 44 receiving placebo. Both rupatadine 10mg and 40mg were found to be safe and did not cause any increase in adverse effects when compared to the placebo. The proportion of individuals who developed either pleural effusions or ascites (22.7%), were similar in all 3 arms. None of the patients given rupatadine 40mg developed bleeding manifestations, while 2 (4.5%) in the 10mg and 5 (11.4%) in the placebo arms developed significant bleeding manifestations. None of the patients in the rupatadine 40mg arm developed organ dysfunction, while 1 (2.3%) patient in 10mg arm and 3 patients (6.8%), in the placebo arm developed liver dysfunction. Those given rupatadine 40mg daily had less reduction in the platelet counts and less elevation of liver transaminases when compared to the 10mg rupatadine and the placebo arm. Rupatadine appears to be safe in patients with acute dengue infection. Although rupatadine did not reduce the proportion of individuals who develop fluid leakage when given on day 4-5 of illness, it appears to reduce complications associated with dengue. However, it will be important to confirm these findings in larger studies.

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COMPARISON BETWEEN DENGUE AND CHIKUNGUNYA BY CBC AT THE HOSPITAL OF THE NO. 2 POLICE OF THE CITY OF GUAYAQUIL PERIOD 2015

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Introduction Dengue and chikungunya are similar in their classical forms of clinical presentation, especially present as febrile syndrome and in areas where both viruses can circulate simultaneously confused clinically and even coexist in the same patient, also transmitted by the same vector. It is important to reach a differential diagnosis in patients with febrile illness as dengue carries a worse prognosis. Objectives This study aims to define and compare the clinical and laboratory associated with each condition in a hospital population who came to the hospital for febrile syndrome Police features N.2 Methodology A retrospective cross-sectional study. 150 patients were compared to positive 150 Chikungunya IgM positive patients Dengue IgM. Laboratory variables were extracted through medical records of patients and laboratory records Health Center, then compared by measuring association adjusted odds ratio obtained by logistic regression. Software used STATA version 14.1 for Mac. results The parameters of

the blood count which most were associated with Chikungunya were leukopenia with an OR 3.5 CI (1.63-7.47) p = <0.000, OR 7.14 IC low Hemoglobin (1.71-29.85) p = <0.000. however not decreased hematocrit IC OR 0.58 (0.27-1.23) p = 0.15 and OR 1.05 plaquetopenia IC (0.41-2.65) p = 0.9 In the case of Dengue low hematocrit OR 1.71 CI (0.8-3.63) p = 0.15, OR 0.13 low hemoglobin IC (from 0.03 to 0.58) p = 0.016, OR 0.94 plaquetopenia IC (0.37-2.38) p = 0.9, leucopenia OR IC 0.28 (0.13 to 0.61) p = <0.000. In the distribution by age was evident that CHIKV only present in the 80 years Dengue change in the most affected range was 20-40 years but was not significant p = 0.38 GPT transaminase CHIKV presents an OR 0.26 IC (0.05-1.22) p = 0.06, OR 3.84 Dengue the IC (0.81-18.05) in the case of GOT in CHIKV OR 0.47 IC (0.10-2.20) p Dengue = 0.33 and OR 2.10 IC (0.45-9.79) p = 0.23 Discussion We found a clear distribution of laboratory values that associate low hemoglobin disorders and leukopenia with Chikungunya, the platelet count and hematocrit was indistinct between the two diseases. T.

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A CLINICAL GRADING AND DECISION SUPPORT SYSTEM FOR DENGUE VIRAL INFECTION AND ACCOMPANYING COMORBIDITIES USING SYNDROMIC SURVEILLANCE IN THE HOSPITAL SETTING

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Dengue fever has increased its geographical spread across non-endemic areas. Therefore, timely surveillance and clinical decision-making before, during, and after outbreaks of dengue are highly important to controlling epidemics. While dengue has not been endemic to Taiwan, outbreaks have occurred annually in southern Taiwan for the vast majority of the past two decades. Therefore, Taiwan's health authorities have employed a system of both passive surveillance and serological surveillance for the detection of dengue. However, as detection of early cases may prove to be challenging using current methods, a more sophisticated form of is preferable for the early detection of cases of dengue, especially as Taiwan's case population is one of the oldest in the world and complicated with multiple co-morbidities such as diabetes and cardiovascular, renal, and liver disease. Extending previous studies from this group, this study attempts to establish a clinical grading and decision support system based around a concept of syndromic surveillance, drawing from a hospital database of confirmed cases of dengue in southern Taiwan and its complete set of electronic health record information to determine the significance and contribution of accompanying co-morbidities on the clinical presentations of dengue infections. The resultant data provide helpful insight into the formulation of clinical guidelines for early detection and management of dengue infection, particularly for severe, complicated cases in adults. Syndromic surveillance in an area non-endemic for dengue such as Taiwan may also prove applicable for other non-endemic nations also under the threat of dengue fever outbreaks.

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INVESTIGATING DENGUE VIRUS INFECTION AS A CAUSE OF FEVER IN KENYAN CHILDREN

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Dengue virus (DENV) is endemic in more than 100 countries around the world and causes 400 million infections yearly. 16% of DENV disease is predicted to occur in Africa, but due to lack of surveillance, the burden of DENV in the Africa remains uncertain. The objective of this study is to describe the incidence of DENV infection in febrile children in coastal and western Kenya. We are enrolling children presenting with acute fever to any of 4 health centers located in Chulaimbo and Obama Children's Hospital in western Kenya, and Msambweni and Ukunda on the Kenyan coastal region. To investigate whether DENV causes febrile illness in Kenyan children, clinical data and blood samples are collected at presentation and at one month follow up and tested for DENV IgG by ELISA. Out of 180 paired serum samples tested to date, 1 (0.6%, 95% CI 0.01 to 3.1%) male subject aged 2 years old seroconverted from Chulaimbo. Based on preliminary results the incidence of DENV infection is 56 per 10,000 cases of febrile illness in Kenyan children.Further ongoing testing of 1000 paired samples will measure the true incidence of DENV infection in this region. These data will be helpful to clinicians since dengue symptoms are non-specific and lack of awareness leads to missed opportunities for community education and vector control and prevention. Without further investigation, our knowledge of the real impact of DENV in the region is severely limited.

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A HUMANIZED MOUSE MODEL FOR STUDYING HUMAN IMMUNOLOGY AND PATHOGENESIS OF DENGUE VIRUS INFECTION

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The limitation in our current understanding of dengue disease pathogenesis may be attributed to the lack of an ideal animal model. To address this, we have utilized a mouse strain, DRAGA, that is immunocompromised and transgenically expresses Human Lymphocyte Antigen (HLA) molecules. We hypothesize that these mice are capable of sustaining dengue virus (DENV) infection and will generate a human immune response to DENV. We conducted experiments to determine if DRAGA mice can support DENV replication, sustain DENV infection, develop clinical signs of disease, and produce a humoral and cellular immune response to DENV. Mice were injected subcutaneously in the right flank with 1x106 pfu of DENV-1 Western Pacific 74, and monitored for several weeks for clinical signs (clinical score, temperature, weight loss). Blood was sampled at various time points for viremia determination by a CDC DENV-1 RT-PCR assay. Measurements of anti-dengue antibody titers (IgM and IgG) in serum samples were done at various time points using two-fold serial dilutions of sera in an in-house ELISA. Cell mediated immune responses in spleens of humanized mice was measured by IFN-γ ELISPOT assay at the time of euthanasia, and organs were harvested for detection of virus. Mice demonstrated relevant clinical symptoms. Clinical scores (including rashes, hunching posture, lack of movement) increased for all five mice resulting in all five mice being euthanized before day 80. Kaplan-Meir results indicate a difference in outcome for infected mice compared to controls (P=0.0011, Log-Rank Test). Viremia was detected in all mice. At the time of euthanasia one mouse demonstrated a strong cellular immune response to DENV non-structural 1 glycoprotein by ELISPOT following stimulation by peptide pools (P=0.0013, Unpaired t test). No other cellular immune response was observed. This mouse also demonstrated an IgM humoral immune response. This model has the potential to represent a powerful small animal model for the preclinical testing of experimental vaccines and become a critical capability for advancing candidate dengue vaccines to human trials.

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EVALUATING INTERNET-BASED COMMUNICABLE DISEASE BIOSURVEILLANCE METHODS FOR VECTOR BORNE DISEASES: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Internet-based vector-borne disease (VBD) surveillance methods using 'big data' sources such as Google and Twitter have recently been developed. These methods may offer complementary real-time VBD surveillance, but appraisal of their performance and validity is essential for their improvement and possible implementation. We performed a systematic review and meta-analysis to assess the performance of VBD internet-based surveillance methods and the quality of evidence supporting them. We included studies that predicted population-level VBD activity with search-engine, social media and other forms of internet data and that were validated against a reference source of public health data. We searched MEDLINE, EMBASE and Web of Science databases in addition to reviewing bibliographies. Study quality was assessed using a framework covering documentation, analysis reproducibility and external validation. Studies measuring performance by correlation between internet-predicted and reported VBD trends underwent meta-analysis, weighted by effective sample size. Subanalyses were performed to explore heterogeneity. Of 2476 non-duplicate studies, 14 met eligibility criteria, of which 11 examined dengue, and the remainder malaria, leishmaniasis, Lyme disease or other arboviruses. Two completely satisfied quality criteria across all domains and six underwent external validation. Ten were included in the meta-analysis, yielding a summary correlation r = 0.69 (95% CI 0.67 - 0.70) with large heterogeneity between subgroups of pathogens, internet data type and disease endemicity. Internet-based VBD prediction performed best for malaria (r = 0.92) and dengue (r = 0.73); and with data derived from Wikipedia (r = 0.89) and Twitter (r = 0.70). Internet-based surveillance is overall moderately accurate for detecting trends in VBD yet may perform better for certain pathogens, internet-data sources and disease burdens. Variable study quality warrants further rigorous study in this field.

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COMPLEMENTARY USE OF CRYO-ELECTRON MICROSCOPY AND MASS SPECTROMETRY TO ASSESS THE MATURITY OF SANOFI PASTEUR DENGUE VACCINE VIRUSES

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The Sanofi Pasteur dengue vaccine demonstrated significant efficacy in phase III studies and is now licensed in several countries. The vaccine is composed of four recombinant, live, attenuated viruses based on a yellow fever 17D vaccine backbone, each expressing the pre-membrane (prM) and envelope (E) genes of one of the four dengue virus serotypes. Dengue is a flavivirus, which are small, enveloped, icosahedral, positive-single strand RNA viruses. The glycoprotein shell consists in 180 copies each of an envelope (E) and membrane proteins (prM/M, prM being M precursor). In immature particles, 60 trimeric spikes extend from the particle surface, each of them consisting of 3 prM:E heterodimers, conferring the "spiky" morphology to the virion as observed by cryo-electron microscopy. Maturation process occurs during transport through the Trans-Golgi where E undergoes conformational changes triggered by low pH, after which prM is eventually cleaved by furin, a host protease. The final mature particle presents 90 E homodimers on its surface and usually presents a "smooth" morphology, while it may also present a "bumpy" structure depending on the temperature and serotype. Maturity plays a critical role in dengue virus infectivity but it remains unclear if it is linked to some extent to immunogenicity and eventually protection triggered by vaccine viruses. To first address this question at the protein level, we have developed a mass spectrometry based method (LC-MS) to quantify specifically prM, pr and M proteins and estimate the mean maturity of the 4 vaccine viruses that were part of clinical phase III studies. Virus maturity was also analyzed by cryo-electron microscopy at the particle level. Percentage of spiky (immature), smooth/bumpy (mature) and mixed (partially mature) viral particles were determined. Results from these two orthogonal methods were in good agreement and showed a significant maturity for all batches and serotypes used in Phase III studies. In this regard, it does not appear that differences in serotype-specific efficacy observed in these trials could be linked to differences in maturity for the corresponding serotypes.

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A STATISTICAL APPROACH TO ESTIMATE DENGUE VIRUS INFECTION HISTORIES, INCLUDING BROADER CRITERIA FOR INAPPARENT INFECTIONS

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The four dengue viruses (DENV1-4) cause 100 million symptomatic dengue cases and an estimated 300 million inapparent infections each year. We explored current assumptions used to identify inapparent infections and describe a statistical approach to create probabilistic infection histories for children in the Nicaraguan Pediatric Dengue Cohort Study (PDCS), ongoing since 2004. Inapparent infections were identified by a ≥4-fold increase in antibody titers as measured with the Inhibition ELISA (IE) between annual samples. Controlling for year, we found that the proportion of children with symptomatic or inapparent infections decreased with higher pre-infection IE titers. Further, there was a significant inverse relationship between pre-infection IE titer and the magnitude of IE titer increases the following year. These observations suggest that children with high pre-infection IE titers may control infection without observable disease *or* large increases in IE titer, making them an important group for studying protection. We also observed that the number of boosts (≥2-fold increases in IE titer) correlated with epidemic cycles: the largest proportion of children had boosts before or after the year with the highest DENV incidence, with the lowest proportion of boosts observed in years of transition in epidemic dominance of one DENV type to another. Consistent with this observation, children with symptomatic infections were more likely to have an increase in IE titer the next year if that year had high incidence of symptomatic dengue, even if the dominant circulating DENV type matched their previous infecting type. Based on these observations, we are estimating probabilistic infection histories for all children in the PDCS with a Bayesian approach that accounts for infections that cause <4-fold IE titer increases as well homologous reinfection. From this model, we estimate the infection probability per child per year and repeatedly sample the cohort with these probabilities to create DENV infection histories. We then use these infection histories to test for determinants of protection against symptomatic dengue and severe disease.

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SUSCEPTIBILITY OF NEOTROPICAL PRIMARY BAT CELL LINES TO DENGUE INFECTION

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Dengue Virus is the most widespread arboviral disease that affects humans worldwide. Bats have been identified as carriers of emerging viral zoonoses and proposed as possible Dengue reservoirs, since viral RNA/NS1 and/or antiviral antibodies have been detected. Yet, Dengue experimental inoculation of Artibeus bat species failed to show dengue replication. Also, a putative organ for virus replication in bats is still not identified. Therefore for testing *in vitro* susceptibility of bat cells for dengue infection, we established primary bat embryonic cells from diverse organs of three different bat species (Artibeus jamaicensis, Molossus sinaloae, and Desmodus rotundus). We observe a serotype-, organ-, and bat species-specific dengue susceptibility of infection, though virus replication in all cases is limited. Only Molossus-derived fibroblasts, kidney, liver, and intestine cells sustained poor dengue serotype 1 replication, though at 96 hpi replication is controlled by bat-specific mediators. Dengue does not replicate efficiently in cell lines derived from the other bat species. Therefore, Artibeus and Desmodus bats may unlikely serve as dengue reservoirs. Nevertheless, to elucidate if Molossus bats play a role in dengue replication, ecological or *in vivo* experiments must be performed, however with an appropriate dengue serotype.

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EFFECTS OF COMMUNITY STRUCTURES AND ENVIRONMENTAL HETEROGENEITIES ON THE SPREAD AND PERSISTENCE OF DENGUE

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Dengue, a multi-strain vector-borne tropical disease, presents a substantial and increasing burden on global public health. The interactions between the virus, its mosquito vectors and the human host are complex and only partially understood. Immune competition between dengue's four antigenically related serotypes (DENV1-4) together with the dependencies of vector ecologies on environmental attributes, such as temperature, rainfall, and host density, introduce strong spatio-temporal heterogeneities, resulting in irregular epidemic outbreaks and asynchronous oscillations in serotype prevalence. Local and global human movement have been implicated as important drivers of dengue epidemiology across space and time and further create the conditions for the geographic expansion of dengue into new habitats. However, the effects of demographic and ecological structures on dengue epidemiology have not yet been explored in detail. To this end, we constructed a stochastic individual-based transmission model that explicitly includes spatio-temporal heterogeneities in host and vector population sizes and further incorporates complex community structures and connectivities between sub-populations. The model's more realistic meta-population formulation allows for the exploration of the effects of environmental heterogeneities on dengue incidence, in addition to the identification of critical community size and connectivity necessary for the emergence and spread of dengue virus. Our results will thus help to better understand the spatio-temporal epidemiology of dengue and assess the risks of further geographic expansion.

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DEVELOPMENT OF ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY ASSAYS FOR DENGUE VIRUS

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Dengue virus (DENV) infection affects US military personnel deployed to endemic areas. DENV infection leads to a number of host immune responses, one of which is antibody-dependent cell-mediated cytotoxicity (ADCC). The aim of this study is to develop and standardize assays to assess serum ADCC activity. We first examined the percentage of antigen opsonization and level of natural killer (NK) cell degranulation using CEM-NKR-DC-SIGN cells as targets and normal human PBMCs as effectors. CEM-NKR-DC-SIGN cells were infected with DENV for 3 and 24-hours. The infected cells were treated with a dengue immune and a control naïve serum at five-fold dilutions and then with a secondary antibody, PE-labelled goat anti-human IgG Fc. The opsonization assessment indicated similar levels of DENV Ag expression on the surface of target cells at both 3- and 24-hours. The degranulation experiment was done by co-incubating the effectors and the opsonized targets for 2 hours and consequently staining the cultures with an antibody cocktail (APC-CD56, PerCP-CD3, FITC-CD107a, and PE-CD16). The expression of CD107a on CD3-CD56+ suggested a significant increase in degranulation of NK cells against target cells infected for 24-hours but not 3 hours for all four serotypes. This could be due to possible increase in expression of different viral antigens on the cell surface at 24-hour. Additionally, cells infected by DENV for 24 hours may express certain stress factors and certain NK cell activation ligands which could potentially increase functional activity of NK cells. ADCC is a mechanism that leads to lysis of infected cells; therefore it is important for viremia control during an infection. Hence we are working towards developing a non-radioactive lysis assay and further standardizing these assays for future application for clinical research and vaccine development.

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SEROCONVERSION TO DENGUE AND CHIKUNGUNYA IN IMMUNOLOGICALLY NAÏVE ADULTS IN ST. KITTS

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Ross University School of Veterinary Medicine (RUSVM) is located on St. Kitts and Nevis, a popular tourist destination in the Lesser Antilles in the Caribbean. Dengue and chikungunya are endemic on St Kitts and students attending RUSVM for their 2 ½ years of study principally come from areas where these diseases are not present. Studying the rates of exposure of the students to the viruses and the risk factors involved would provide valuable epidemiological data for students and tourists visiting areas where dengue and chikungunya are endemic. The aim of the prospective study is to determine the survival time and risk factors in naïve adults visiting an endemic area. Student volunteers attending RUSVM at one of the three intakes between September 2014 and May 2015 were recruited for the study. Whole blood was collected from consenting volunteers when they enrolled in the study and every 4 months subsequently. Plasma was separated and tested with a DENV IgM and IgG antibody capture ELISA developed at the Centers for Disease Control and Anti-Chikungunya Virus ELISA IgM test and Anti-Chikungunya Virus ELISA IgG test - (Euroimmun, Lübeck, Germany). Plaque reduction neutralization (PRNT) following standard methods is also being performed to confirm the dengue serology results. A total of 161 of the 218 volunteers were sampled within 4 weeks of arrival to the island with 76 (47%) already showing IgG antibodies to dengue virus at titers of 2 or higher. Of these, 27 reported they had not previously visited any dengue endemic areas which would indicate that seroconversion may have occurred very shortly after arrival on St Kitts. In

the subsequent testing of the students we found 21 (10%) seroconverted against dengue virus with an average time to seroconversion of 24 weeks. The rate of seroconversion between sampling periods was 1.35 per 10 individuals. One student (1%) seroconverted against chikungunya within 2 weeks of arrival while another 4 (2%) seroconverted subsequently with an average time to seroconversion of 25 weeks. None of the seropositive students reported classical signs of dengue or chikungunya. (The confirmatory PRNT results for dengue are pending).

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FLUORESCENTLY LABELED FLAVIVIRUSES TO TRACK ANTIGEN-SPECIFIC B CELLS

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We recently developed alexa fluor labeled dengue viruses (AF DENV) to evaluate frequencies of antigen-specific memory B cells in the peripheral blood of immune individuals. We used two serotypes of AF DENV together on PBMC from children in Thailand undergoing acute primary or secondary DENV-1 infections to determine whether patterns emerged on antigen-specific B cells that reflected their exposure or clinical diagnosis. Brightly labeled AF DENV serotype specific and cross-reactive B cells were identified in PBMC from all subjects. Frequencies of AF-DENV+ class switched memory B cells (IgD-CD27+ CD19+ cells) reached up to 8% during acute infection and early convalescence. In a number of subjects, AF DENV labeled B cells expressed high levels of CD27 and CD38 during acute infection, characteristic of plasmablasts, and transitioned into memory B cells (CD38-CD27+) at the early convalescent time point. Our analysis of total B cells and AF DENV+ B cells revealed higher activation of memory B cells early during acute secondary infection suggesting reactivation from a previous dengue infection. AF DENV are useful reagents to identify differences in the phenotype of subsets of antigen-specific and cross-reactive B cells during and after natural dengue infection and vaccination.

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A CASE OF DENGUE ENCEPHALITIS CAUSED BY DENV-4

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We describe a case of dengue encephalitis in a primary dengue infection caused by DENV-4. A 62 year old Chinese woman, previously well, presented with a four-day history of fever, headache, postural giddiness, vomiting and exertional dyspnoea. She had not travelled out of Singapore. Clinical evaluation on admission (day 4 of illness) revealed temperature of 38.2oC, blood pressure 140/80 mmHg, pulse rate 92/min, physical examination was otherwise unremarkable. Total white cell count was 1.9 x 109/L (neutrophils 1.2 x109/L), haemoglobin 13 g/dL, haematocrit 39.5%, platelet count 106 x 109/L, ALT 218 u/L and AST 155u/L. Renal function was normal. Dengue NS1 antigen was positive, dengue IgM and IgG were negative. Chest radiograph was normal. She developed confusion and expressive aphasia on day 6 of illness. Neurological exam was unremarkable with no focal deficit. Blood cultures were negative and there was no pyuria. Cerebrospinal fluid (CSF) analysis revealed a cell count of 62 cells/uL (97% lymphocytes), red cell count 9 cells/uL, protein 2.33 g/L and glucose 3.5 mmol/L (serum glucose 5.9 mmol/L). CSF bacterial culture, antibody (measles, mumps), and polymerase chain reaction (PCR) for CMV, HSV, VZV, Toxoplasma, Enterovirus were negative. Magnetic resonance imaging of the brain was normal. CSF dengue PCR was negative but IgM and IgG were positive. Serum dengue PCR was positive for DENV-4. She was treated with intravenous acyclovir till PCR results were available, and made a complete recovery at day 11 of illness. The proposed case definition for dengue encephalitis includes (1) fever, (2) acute cerebral involvement, (3) positive dengue IgM or PCR on serum and/or CSF, and (4) exclusion of other causes of viral encephalitis, encephalopathy as demonstrated in our patient. Prior case series of dengue encephalitis have been associated most commonly with DENV-2, DENV-3 and occasionally with DENV-1. ,3 Although DENV-4 has been reported to potentially cause encephalitis, those cases were fatal with multi-organ involvement and dengue haemorrhagic fever , . This is the first known case of DENV-4 encephalitis with complete recovery.

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PROFILING ANTIBODY RESPONSES TO DENGUE NS1 IN VACCINE STUDIES AND NATURAL HUMAN INFECTIONS USING PEPTIDE MICROARRAYS

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A major challenge in dengue vaccine development is that cross-reactive anti-dengue virus (DENV) antibodies can be protective or potentially enhance disease via antibody-dependent enhancement. DENV nonstructural protein 1 (NS1) has long been considered a vaccine candidate, and we have shown that NS1 from all 4 DENV serotypes protects against challenge in a mouse model of lethal vascular leak syndrome. Conversely, we found that DENV NS1 by itself triggers vascular leak *in vivo* and *in vitro* and increases disease severity during infection. Here, we evaluated survival to challenge in the lethal DENV vascular leak model in mice immunized with NS1 combined with alum, Monophosphoryl Lipid A (MPLA) + AddaVax, or Sigma adjuvant system (SAS) + CpG DNA. We characterized antibody responses to NS1 using a microarray with 20-mer peptides (overlapping by 15 amino acids [aa]) from prM, E, and NS1 of DENV-1, -2 and -3. We compared these antibody profiles to those of mice immunized with ovalbumin (OVA) plus adjuvant and mice infected with a sublethal dose of DENV2. Mice immunized with OVA or NS1+alum were not protected, whereas immunization with NS1+MPLA/Addavax or NS1+SAS/CpG or prior infection with DENV2 resulted in 100% survival but exhibited distinct antibody responses to NS1 peptides. We identified two DENV-2 NS1 peptides (D345 and D347) that were recognized strongly by NS1+MPLA/Addavax-immunized mice and DENV-infected mice; analogous peptides in DENV-1 and -3 were similarly recognized. In addition, immunization with NS1+MPLA/AddaVax induced antibodies specific NS1 epitopes that were not observed following DENV2 infection (e.g, D356). In parallel, we found that human sera from natural DENV infections reacted to the same D345 and D347 peptides but did not react to D356. We mapped these epitopes onto the NS1 crystal structure and found that D345 and D347 mapped to aa 101-130 in a surface-exposed flexible loop in the wing domain. D356 (aa 156-174) is in the putative membrane-binding "greasy-finger" region of NS1. These data identify antibody responses to NS1 vaccination that target specific epitopes and may help prevent NS1-mediated pathogenesis.

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POLYCLONAL ANTIBODY RESPONSES TO SEROTYPE-SPECIFIC NEUTRALIZING EPITOPES IN NATURAL DENGUE VIRUS INFECTIONS

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The development of an effective and safe dengue vaccine relies on identification of neutralizing epitopes from all four dengue virus (DENV) serotypes. Strongly neutralizing DENV serotype-specific human monoclonal antibodies (hmAbs) target quaternary epitopes spanning multiple E protein monomers and are only preserved in intact virions. Among hmAbs with these properties is 5J7, which is a DENV3 serotype-specific hmAb. Given the potential role of the 5J7 epitope as a determinant of the DENV3 type-specific neutralizing response, we previously created a partial functional transfer of the DENV3 5J7 epitope into a DENV4 background. The recombinant virus, rDENV4/3, is neutralized by human DENV3-immune sera, indicating the functionality of this epitope. Here, our study aims to explore the recognition of the hmAb 5J7 epitope in the epidemiological context of an endemic setting. Specifically, we are assessing the proportion of 5J7 epitope-specific neutralizing antibodies following primary DENV3 infection and determining the kinetics and magnitude of the response over time. We first analyzed primary DENV3 sera from 24 individuals enrolled in a longitudinal dengue hospital-based study in Nicaragua, which allowed us to track the proportion of the DENV3 type-specific neutralizing response attributable to the 5J7 epitope at two time-points post-infection (3 and 18 months). The neutralizing titers (NT_{50}) to the rDENV4/3 virus and its parental viruses (DENV3 and DENV4) were obtained via a flow cytometry-based neutralization assay using human U937-DC-SIGN cells. We also measured the cross-reactive DENV4-directed antibody responses that recognize the rDENV4/3 virus at 3 and 18 months post-infection. Further studies will evaluate how the DENV3 type-specific neutralization tracks with the 5J7 epitope using samples from our Nicaraguan cohort study over multiple years and following second and third DENV infections. Overall, this project contributes to increased understanding of the antibody response in natural DENV infections and has direct implication for design and evaluation of dengue vaccine candidates.

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ENDURING A DENGUE/ZIKA EPIDEMIC IN RIBEIRAO PRETO, BRAZIL

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Dengue fever (DF) is the most important arbovirus infection in the world. Our busy hospital emergency room (ER) located in an endemic DF region in Brazil has been facing large DF outbreaks every year. In 2016, a new virus (Zika) was introduced to our region. We described how we dealt with both epidemics in the setting of not having Zika serology and reserving Zika RT- PCR for pregnant women (PW). The patient care flow was based upon in the Lean Methodology that has a focus in process improvement with the identification and elimination of everything that does not add value. We followed the Brazilian Health Ministry Guidelines for dengue care; the focus was prompt detection of alarm signs, vigorous endovenous hydration for high hematocrit and low platelet count, oral hydration for every patient at the ER and daily or every other day returns based upon the patient risk to develop severe DF. We established a flow for PW presenting with a rash and/or suspected DF (SDF); they would have daily returns, samples would be sent to Zika RT- PCR testing and dengue testing, and after disease resolution, they would be followed at a high-risk pregnancy clinic (HRPC). From January 1 to April 10, 2016, the total # of SDF patients was 29, 841, 43% of the total # of ER patients (69,777); the peak of SDF patients was seen on February (12,668). Only 101 patients (0.34%) of SDF were admitted to the hospital; there was one death of a woman with adrenal insufficiency. Eight hundred thirty seven NS1 tests were done, with 120 (14%) positive. One hundred twenty six PW presented to the ER with rash and/or SDF; six were NS1positive, 23 with DF IgM; 22 (17% of total) had Zika RT-PCR results released by the State Reference Laboratory for Zika (13 positive and 9 negative). There were two spontaneous abortions in both groups; 91 women (72%) are still seen at the HRPC with no report of microcephaly so far. DF disrupts ER routines in our region; it is possible that many SDF patients had in fact Zika but because tests were not available except for PW, all were treated as SDF. Health services in our region need to be prepared to endure dengue and Zika epidemics in the next years.

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ACCESSING HEALTH CARE IN VENEZUELA: A COMMUNITY BASED STUDY ON HEALTH CENTER ATTENDANCE FOR DENGUE AND FEVER

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Dengue is a major public health problem in Venezuela. Timely health centre (HC) attendance is crucial in reducing mortality and severity of dengue. The health care system in Venezuela comprises a public and a private sector. The public system includes the traditional primary/secondary (Ambulatorios), and tertiary level HCs (Hospitals) and is usually free of charge. To improve limited access to care for the poorer, a parallel public health care system ("Mission Barrio Adentro") was set up in 2003. We assessed the intended HC attendance in the case of fever and suspected dengue in an urban area of high dengue transmission. Between September 2013 and February 2014 a crosssectional household survey was performed in Maracay, Venezuela. Intended HC attendance and the perceived barriers in the case of fever and dengue of adults and parents/guardians of children were assessed. Data was collected through structured questionnaires from 105 individuals. We show that people would visit several different HCs if needed, and that the health preferences differed throughout the community. The most frequent first choice of health centre was an Ambulatorio, in the case of fever (n=82; 78.8%) and dengue (n=84; 80.8%). Several economic, ethnic, logistic, and quality aspects influenced the preference to access the HCs. Individuals preferred to first attend traditional HCs as they trusted the care given at these institutions, but a barrier was the lack of treatment supplies. Although the lack of supplies was mentioned to a lesser extent in the case of the parallel HCs, people reported not to trust the medical staff, nor the diagnosis and treatment given in these HCs. Furthermore, the private care, which was considered best, was mainly accessible for those with a health insurance. A higher education (fever/dengue: p=0.001/p=0.001) and a nonmanual occupation (fever/dengue: p=0.007/p=0.016) were associated with more intended private HC attendance. Access to care in Venezuela is currently a complex situation where individuals need to juggle between the different available public and private HCs in order to obtain proper/timely care and medical supplies.

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IDENTIFICATION OF CLINICAL AND LABORATORY PARAMETERS THAT DISTINGUISH BETWEEN DENGUE AND NON-DENGUE ILLNESS WITHIN THE FIRST 72 HOURS OF FEVER

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As of March 2016, the prospective multicentre IDAMS study has enrolled 7178 out-patients aged ≥ 5 years in 8 countries across Latin America and Asia. Participants with non-specific febrile illness are enrolled within 72 hours of fever onset and followed daily with a common protocol, a key objective being to identify simple clinical and/or laboratory parameters that distinguish between dengue and other febrile illness (OFI) thus leading to an evidence-based case definition. Laboratory diagnosis relies on an algorithm including PCR, NS1, and IgM seroconversion, all performed following strict protocols. We will analyse clinical and laboratory parameters at enrolment as predictors for confirmed dengue by multivariable logistic regression and flexible classification algorithms. The full analysis will be carried out stratified by age group, day of illness and country/ continent, and subsequently pooled if appropriate. To improve model prediction we will also include changes between enrolment and the following day, thus basing the assessment on two time points. Data is currently available on 5078 Asian participants originating from Vietnam, Malaysia, Cambodia, Bangladesh and Indonesia, with data from Latin America (Brazil, Venezuela, and El Salvador) due to be added after the study closes in June 2016. In the preliminary pooled analysis, adjusting for age group and day of illness at enrolment, the presence of skin rash and skin flush, anorexia, dizziness, diarrhoea and conjunctival injection at enrolment was associated with a diagnosis of dengue. The strongest association was found for skin bleeding and low platelet count at enrolment. The presence of sore throat, cough, and rhinitis was associated with OFI. However, more than 20% of patients with these symptoms had laboratory confirmed dengue, highlighting the role of dengue in the differential diagnosis when upper respiratory tract symptoms are present. The findings of the full analysis will be presented and are expected to have an important impact on diagnostic algorithms for dengue in settings where confirmatory laboratory testing is not possible.

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THE ASSOCIATION BETWEEN DENGUE PRE-EXISTING ANTIBODY ON ZIKA VIRUS INFECTION IN THP-1 MONOCYTES CELL LINE

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Antibody-dependent enhancement of infection (ADE) is postulated as one factor contributing to dengue severe disease such as dengue haemorrhagic fever and dengue shock syndrome (DHF/DSS). Pre-existing antibody against heterotypic dengue serotypes binds to the virus but does not neutralize it. Thereby, enhancing DENV infection of Fcγ receptor bearing cells increasing the number of viral infected cells and the amount of virus produced. DENV has routinely showed cross-reactivity in serological assays with Zika virus (ZIKV) and co-infections of DENV and ZIKV have been reported. Here we report the effect of pre-existing DENV antibody on the ability of three different strains of ZIKV (African and Asian genotypes) to infect THP-1 monocytes cell line. The mean amino acid sequences homology between DENV and ZIKV showed 42.9%, 42.5% and 57.0% identical for capsid, prM and E regions, respectively. The growth of ZIKV in the presence of pre-existing DENV antibody was higher than that in the absence of DENV antibody. Immune mediators involved virus infection were also investigated. ADE-induced ZIKV infection had higher production of IL-12, a pro-inflammatory cytokines than in the absence of DENV antibody. Down-regulation sensors including RIG-I and MDA-5 are waiting to be elucidated. As DENV vaccines have been licensed in endemic areas of both DENV and ZIKV, an understanding of the pre-existing DENV antibody effect on other flavivirus is necessary.

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PREPARATION OF STANDARD ZIKA VIRUS (ZIKV) IGM FOR SEROLOGICAL DIAGNOSIS OF ZIKV INFECTION

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Laboratory diagnosis of acute Zika virus (ZIKV) infection during the febrile phase of illness currently depends on detection of the virus by RT-PCR. A specific IgM serological assay for ZIKV has yet to be developed. Here we describe the development of a specific anti-ZIKA IgM ELISA for the diagnosis of acute ZIKV infections. To develop a specific anti-ZIKA IgM antibody to use as a positive control for the assay, the ATCC strain of ZIKV was subcutaneously injected into a Rhesus monkey at a dose of 5x106 PFU. Blood was collected from the monkey daily from 0 to 12 days after infection and on days 15 and 30. Viremia was determined by RT-PCR and IgM/IgG antibody kinetics was determined by EIA form blood collections. The data show ZIKV was detected by PCR on days 1, 2, and 3 with the highest peak occurring on day 2. ZIKA specific IgM was detected starting on day 6 and continued through day 30 with a peak from days 10 to 15. To determine the specificity of the assay we tested known positive cases of DENV, JEV and ZIKV to establish the amount of cross-reactivity from previous infections. The following samples were used to test the specificity of the ZIKA ELISA: 9 cases of primary DENV, 10 cases of secondary DENV infection, 10 cases of JEV infection, 3 cases of ZIKV infection and 7 cases of negative specimens. The cut off value for the anti-ZIKV IgM/IgG EIA was set at ≥40 EIA units. The results showed that primary DENV infection and samples testing negative for other flaviviruses tested negative for anti-ZIKA IgM. However, 1 out of 10 secondary DENV infections and 2 of 10 JEV infections tested positive for ZIKV IgM most likely due to cross-reactive antibodies. These data indicate that detection of acute ZIKV infections in patients previously exposed to flavivirus could produce false positive results under certain conditions but should be useful in detecting ZIKV in patients with primary flavivirus infections.

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CO-CIRCULATION OF ZIKA, CHIKUNGUNYA AND DENGUE VIRUSES DURING DENGUE OUTBREAK IN SUMATRA, INDONESIA

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Arthropod-borne viruses (arboviruses) are known to cause significant public health problems throughout the world. Indonesia has been affected by the arboviral diseases such as dengue for decades with frequent epidemic cycles. In 2015, dengue cases increased significantly in Jambi municipality in Sumatra with the number doubled from previous year. To understand the dynamic of the disease, we conducted dengue molecular study in Jambi. Sera were collected from dengue-suspected patients and dengue diagnosis was performed using NS1, IgG/IgM, and RT-PCR detection. Of 210 dengue-suspected patients, 107 were confirmed dengue based on NS1 and RT-PCR. All four dengue virus (DENV) serotypes were detected with DENV-1 as the predominant serotype (66%). To determine the disease etiology of the 103 dengue-negative cases, we screened the samples using RT-PCR for other viruses which include flavivirus and alphavirus families. Among them, we detected eight cases were infected by Chikungunya viruses (CHIKV) and one by Zika virus (ZIKV). All viruses were successfully isolated and propagated in tissue culture. The clinical manifestations of the Chikungunya and Zika patients were mild and mimicking dengue symptoms. To determine the genotypes the viruses, we sequenced the Envelope, E1, and NS5 genes of DENV, CHIKV, and ZIKV, respectively. Phylogenetic analyses revealed the DENV-1 viruses belonged to Genotype I, DENV-2 was of Cosmopolitan genotype, DENV-3 as Genotype I, and DENV-4 belonged to Genotype II. The CHIKV isolates were grouped as Asian genotype, while the ZIKV was classified as Asian lineage. Our finding demonstrates the co-circulation of multiple arboviruses during dengue outbreak in Indonesia. This co-circulation will likely to contribute to a large neglected disease burden and causes misdiagnosis and underreporting of these diseases. It is essential that systematic surveillance be implemented to evaluate and monitor the distribution these arboviruses infections and its potential public health problems in Indonesia.

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FROM DENGUE TO ZIKA—TAIWAN'S EXPERIENCE

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Dengue and Zika viruses involve common vectors - *Aedes* (Ae.) aegypti and Ae. albopictus - both widespread in Taiwan. While the epidemics of dengue in Kaohsiung and Tainan in 2015 have been the largest and most severe, one imported Zika case from Thailand was detected in January 2016, indicating that future public health threat of Zika virus can't be ignored. Epidemiology of dengue in Taiwan involves three types: (1) sporadic cases in Taipei where only Ae. albopictus are present, (2) an epidemic type in Tainan with a lower ratio of *Ae. aegypti* /Ae. albopictus, and (3) DENV-endemicity in Kaohsiung with higher ratio of *Ae. aegypti*/ Ae. albopictus. The specific aims of this study were: (1) to compare epidemiology of dengue in Taipei, Tainan, and Kaohsiung, (2) to investigate the impact of daily meteorological factors on mosquito populations and dengue cases, and (3) to address recommendations for prevention and control measures. Among 43,784 total laboratory-confirmed dengue cases in 2015, 43,419 were indigenous cases (99.16%). Most of them were in Tainan (22,777 cases, 52.4%) [predominantly dengue serotype 2 virus (DENV-2)] and Kaohsiung (19,784 cases, 45.6%) [predominantly DENV-1 in the beginning but subsequently turning to DENV-2]. Taipei had 128 confirmed cases, in which 70 were indigenous DENV-2 cases (0.2% of total in Taiwan), with 21 cases from Kaohsiung, and 46 cases from Tainan. About one month after typhoon Soudelor hit, dengue cases of DENV-2 peaked in Tainan, while Kaohsiung still had mostly DENV-1 cases. Interestingly, after the 3-month window period of cross-protection of different serotypes, DENV-2 cases started to climb and peaked about one month after typhoon Dujuan. Taipei, however, with Ae. albopictus only, was least impacted by the typhoon. In conclusion, *Ae. aegypti* plays the most crucial role in dengue transmission, so reducing the population of *Ae. aegypti* is the key element in preventing dengue and Zika infections. The lessons from dengue epidemics in southern Taiwan in 2015 provide an important future direction for the elimination of Ae. aegypti.

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POTENTIAL USE OF SALIVA SAMPLES FOR DIAGNOSIS OF ZIKA VIRUS INFECTION

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Zika virus (ZIKV) is a mosquito-borne flavivirus first isolated in Uganda, in 1947. Since then, sporadic cases of human ZIKV infections were reported in Africa and Asia, but the first ZIKV outbreaks occurred in the last decade, in the Pacific Ocean region. Late in 2014, large outbreaks of acute exanthematous illness (AEI) were reported from various Northeast states of Brazil, and, in April 2015, ZIKV was identified as the etiologic agent. ZIKV diagnosis is challenging, because serological methods is not specific as a consequence of IgM cross reactivity between Flaviviruses. Currently, molecular techniques, such as conventional or real time reverse transcriptase–polymerase-chain-reaction (qRT-PCR), are the most used methods to diagnosis ZIKV. ZIKV RNA is usually detected by RT-PCR in serum samples, but use of alternative samples has already been described. ZIKV RNA has been found in saliva in concomitance with either blood or urine. There are also a few studies describing viral RNA amplified only from saliva. The objective of this study was to investigate the potential use of saliva samples as an alternative for diagnosis of ZIKV infection. In June 2015, nine patients assisted in an emergency health unit of Salvador, Brazil due to an AEI suggestive of ZIKV had both saliva and serum samples collected after two to five days of symptoms onset. Samples were subjected to RNA extraction using the QIAamp Viral RNA Mini Kit (QIAGEN) and ZIKV qRT-PCR described by Lanciotti et al (2008) using the QuantiTect Probe RT-PCR Kit (QIAGEN). Zika RNA was detected in four of nine samples of saliva (Ct values < 38.5). All serum samples were negative. Our findings coincide with that of prior studies and suggest that qRT-PCR performed in saliva samples may have greater sensitivity compared to serum. In addition, obtaining saliva is easier than serum, particularly in newborns or in remote places where medical facilities are lacking. Further studies with larger number of specimens are needed to confirm our findings, but given the current evidence we suggest that in situations where a blood sample cannot be collected, the use of saliva should be considered.

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THE REEMERGENCE OF ZIKA VIRUS: FROM ARTHROPOD VECTOR TO POSSIBLE SEXUAL TRANSMISSION

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Zika virus, first described in central Africa in the late 1940s, has attracted considerable attention in recent months. Over more than six decades, researchers have carefully described its arthropod-mosquito vector, its pathogenicity, and its structure. During the past year, however, numerous studies have suggested the possibility of non-arthropod transmission via sexual intercourse. Indeed, health authorities have cautioned women in high-risk areas to avoid pregnancy because of the risk of fetal microcephaly. Sexual transmission of Zika virus, of course, is quite plausible. Clearly, a number of viral pathogens, most notably HIV, can be transmitted via sexual intercourse. This study will analyze what is—and has been—known about sexual transmission of Zika virus. It will also examine ongoing public efforts to prevent its transmission.

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SERODIAGNOSIS OF ZIKA IN TRAVELERS RETURNING FROM FLAVIVIRUS-ENDEMIC REGIONS

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Zika virus (ZIKV) has disseminated throughout Latin America and is a major public health concern due to an association with adverse pregnancy outcomes such as miscarriage and microcephaly as well as an increased incidence of Guillain-Barré syndrome. Many of the urgent needs (including diagnostics, surveillance, vaccine development, and pathogenesis studies) in responding to this epidemic hinge on a clear and detailed understanding of the human antibody (Ab) response to ZIKV. It is well known that humans produce flavivirus cross-reactive Ab following natural infection and that these responses can confound interpretation of serology during a secondary flavivirus infection - a highly relevant phenomenon in Latin America where dengue (DENV) seroprevalence can exceed 90%. Serodiagnosis is particularly essential in the ZIKV epidemic as the window for diagnosis by molecular methods is narrow and the majority of new infections are asymptomatic. To address these issues and develop critical tools for study of humoral immunity to ZIKV, we recruited travelers reporting potential exposure to arbovirus infection and tested serum samples for reactivity to DENV and ZIKV. Depending on an individual's travel history and country of origin, we find a variety of patters. Some sera samples bind and neutralize a single virus; others demonstrate broad cross-reactivity. Several primary ZIKV infections were identified in those reporting an acute febrile illness during or after travel to Brazil or Colombia in 2015. This well-characterized set of sera serve as a standard of comparison for novel serodiagnostics that we are developing and deploying to ZIKV-endemic regions. Human monoclonal Ab derived from memory B cells of participants with primary ZIKV infection will be used to map binding determinants of ZIKV-specific and flavivirus cross-reactive Ab and define epitopes targeted by neutralizing Ab.

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GUILLAIN-BARRÉ SYNDROME OUTBREAK - BAHIA STATE, BRAZIL, 2016

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In mid-2015, reports of Guillain-Barré syndrome (GBS) increased in certain regions of Brazil. These reports coincided with the introduction and rapid spread of Zika virus in Brazil, and geographic areas with the highest reports of Zika-like illness and GBS overlapped. The Brazil Ministry of Health and CDC performed an investigation to identify risk factors and potential infectious pathogens associated with GBS. We conducted a case-control investigation in the Salvador metropolitan area, Brazil. We defined GBS cases according to the Brighton Collaboration criteria. Two controls matched by age group were randomly selected from the same neighborhoods as the cases using modified WHO cluster survey methodology. We conducted in-person interviews to obtain risk factor and exposure (environmental, food/water) histories in the 2-month period prior to GBS-case onset. Of 77 suspected GBS case-patients, 50 (65%) met Brighton case definition criteria. The incidence of GBS during April-July 2015 was approximately 12-times higher than expected. Among 41 enrolled GBS case-patients and 85 controls, there were no differences in demographic or exposure data. A higher proportion of GBS cases compared to controls reported an antecedent illness (88% versus 21%, P<.01), with rash and conjunctivitis being reported by 71% and 56% of GBS case-patients, respectively, versus 39% and 22% of the controls (P<.01). Arboviral testing is underway for serum samples collected from all cases and controls. Our investigation identified increased incidence of GBS case-patients occurring in tight geo-temporal clustering in the Salvador area during mid-2015. Many GBS case-patients reported an exanthematous illness during a time of recognized Zika transmission in Salvador, suggesting a possible association between GBS and Zika virus infection. Further surveillance for GBS, additional case control studies, and refined Zika virus laboratory diagnostics are needed to substantiate this possible association.

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THE DIFFERENTIAL IMPACT OF YELLOW FEVER VACCINE ACROSS TRANSMISSION CYCLES: ACCOUNTING FOR HERD IMMUNITY IN THE FACE OF ZOONOTIC TRANSMISSION

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Yellow Fever (YF) is a mosquito-borne flavivirus infection, its major burden is concentrated in sub-Saharan Africa. Two transmission cycles co-exist: a sylvatic cycle in non-human primates causing zoonotic spill-over infections in humans and an urban cycle perpetuated in human populations, with intermediate transmission among both humans and non-human primates also playing a role. The relative contribution of these cycles to the human burden of YF is not well understood. After a period of re-emergence, implementation of large mass vaccination campaigns started in 2006 in the most affected West African countries. Mathematical modelling is currently used to inform these vaccination activities. However, vaccine impact estimates differ depending on assumptions about transmission cycles. In urban transmission, herd immunity effects increase the impact of vaccination compared to the sylvatic cycle. To address this issue, we constructed two alternative versions of a model estimating YF burden,

assuming either 100% zoonotic or 100% inter-human transmission. Both models were fitted to reported outbreaks from 1984-2013 using environmental and demographic variables across the endemic zone of Africa, and were calibrated with serological survey data. Over the 1984-2013 period, both models estimated a very similar cumulative YF burden across Africa. However temporal trends in the burden differed between both models. Notably, the inter-human model estimated a lower burden than the zoonotic model for the period corresponding to recent vaccination activities (2006-2013). Over this period, vaccination was estimated to have prevented 4.5x105 (95% CI: 1.5x105 - 10.4x105) deaths according to the zoonotic and 21x105 (95% CI: 8x105 - 45x105) according to the inter-human model. This corresponds to an estimated 2.2 (95% CI: 0.7 - 5.0) and 10.0 (95% CI: 4.0 - 22) deaths averted per 1,000 vaccine doses, respectively. Integrating herd immunity into the model thus strongly influences vaccine impact estimates. Further efforts to assess the relative contribution of both transmission cycles are needed.

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FULL GENOME SEQUENCING OF ZIKA VIRUSES USING A TARGETED AMPLIFICATION APPROACH

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The recent outbreak of Zika virus highlights the importance of having access to sensitive molecular tools for diagnosis and identification of circulating viral strains. Next-generation sequencing, and shotgun sequencing in particular, are powerful tools that can be used to generate complete viral genomes even in the absence of any a priori knowledge about the virus of interest. As such, shotgun sequencing is usually one of the first tools utilized to generate complete viral genomes from clinical samples. Unfortunately, viruses in clinical samples can be present in small amounts, and as such, these "low viral load" samples are often plagued by high signal-to-noise ratios that prevent the generation of complete viral genomes. One way around this issue is to culture the virus in order to increase viral load. However, passaging viruses through laboratory cell lines can have the unintended consequence of introducing mutations that are not observed in the wild. Here, we report a set of 22 Zika-specific primers that can be used to generate overlapping amplicons by PCR to cover the entire Zika genome even in samples with low viral load. Given that PCR amplification efficiency is directly related to template concentration and to amplicon size, samples where Zika is present in low abundance can be fully covered by as many as 11 overlapping segments (of about 1 KB each), whereas samples with high Zika abundance can be fully covered by as few as 5 (of about 2.5 KB each). An added advantage of this targeted amplification approach is that although the overlapping amplicons can be used as starting material for next-generation sequencing libraries, sequencing can also be completed using Sanger methods. As such, this approach may be useful for epidemiological surveillance in low resource settings that may not have access to the latest next-generation sequencing technologies.

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ZIKA VIRUS PERSISTENCE IN BODY FLUIDS AMONG PATIENTS WITH ZIKA VIRUS INFECTION IN PUERTO RICO

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Little is known about the presence or duration of Zika virus (ZIKV) in body fluids other than blood. Understanding the presence and duration of ZIKV detection in urine and saliva may facilitate diagnostic testing. Information about ZIKV in semen and vaginal secretions is urgently needed to inform prevention messages to prevent sexual transmission. Moreover, the relationship between development of anti-ZIKV antibodies and the presence and duration of ZIKV in body fluids other than blood has not been described. To investigate the persistence of ZIKV in body fluids and its relation to immune response, we are conducting a prospective cohort study of patients with laboratory-confirmed ZIKV infection. Patients with rash, fever, arthralgia, or conjunctivitis that present to an out-patient clinic or hospital emergency department in Ponce, Puerto Rico will be offered participation in the study. For those that consent, demographic and clinical characteristics will be collected in addition to blood, nasopharyngeal, and urine specimens. Patients in which ZIKV nucleic acid is detected by RT-PCR in any specimen will be invited for follow-up, in which blood, saliva, and urine specimens will be collected; among participants 21 years and older, semen or vaginal secretions will also be collected. All specimens will be tested for the presence of ZIKV nucleic acid by RT-PCR, and positive specimens will be further tested for virus isolation to evaluate the presence of infectious virus. Each body fluid will be collected on a weekly basis for 4 weeks and biweekly thereafter until two consecutive negative RT-PCR results are obtained from all specimens. Irrespective of RNA detection, body fluids will also be collected for RT-PCR at 2, 4, 6 and 9 months to investigate intermittent shedding. All blood specimens will also be tested by anti-ZIKV IgM and IgG ELISA. Results will be used to update relevant counseling messages and recommendations from the CDC. At the conference we will present preliminary results regarding the antibody response and RNA persistence in body fluids. Only results for body fluids for which sufficient sample size and follow-up will be presented.

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EPIDEMIOLOGIC INVESTIGATIONS OF GUILLAIN-BARRÉ SYNDROME INCLUDING CASE-CONTROL INVESTIGATION TO DEFINE ASSOCIATION WITH ZIKA VIRUS INFECTION — PUERTO RICO, 2016

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Guillain-Barré syndrome (GBS) is a post-infection autoimmune disorder characterized by motor weakness, sensory abnormalities, and/or autonomic dysfunction due to peripheral nerve damage. In February 2016, Puerto Rico Department of Health (PRDH) reported its first case of GBS with evidence of Zika virus (ZIKV) infection. Given reported increased GBS incidence in ZIKV-affected regions, PRDH aimed to calculate GBS incidence, implement a GBS surveillance system, and investigate the association between GBS and ZIKV infection. Medical records at 8 hospitals for patients admitted with suspicion of GBS during 2012-2015 were evaluated using Brighton Collaboration criteria for GBS diagnostic certainty. Of 140 patient records reviewed, 61 (44%) met the confirmed case definition (Brighton levels 1-3). By applying the proportion of confirmed cases to 2013 Puerto Rico medical insurance claim data, 2013 incidence was estimated at 1.6 cases per 100,000 population. For GBS surveillance, neurologists and hospital infection control staff report suspected cases using a case report form and submit specimens for ZIKV diagnostic testing by reverse transcription-polymerase chain reaction (RT-PCR) and anti-ZIKV immunoglobulin M (IgM) antibodies by enzyme-linked immunosorbent assay (ELISA). During January 1-April 11, 2016, 14 suspected GBS cases were reported, of which 6 (43%) had anti-ZIKV IgM antibodies in serum. Of these, median age was 42 years (range = 32-68) and 3 (50%) were male. All 6 patients were residents of ZIKV-affected municipalities in eastern Puerto Rico. A prospective case-control investigation is underway whereby cases are matched to two controls by age and location of residence. Data on behaviors, exposures, and recent illnesses are collected; RT-PCR and IgM ELISA is conducted on blood specimens, and RT-PCR on urine and saliva specimens. Results of the case-

control investigation are pending. The 2013 GBS incidence provides a baseline to assess trends in GBS cases reported to PRDH during ongoing ZIKV transmission. The case-control study will prospectively recruit cases to better define the association between GBS and ZIKV infection.

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CHARACTERIZATION OF ZIKA VIRUS INFECTIONS AND THE POTENTIAL EFFECT OF PRIOR DENGUE VIRUS EXPOSURE IN CHILDREN IN MANAGUA, NICARAGUA

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The first evidence of Zika virus (ZIKV) emergence in the Americas was documented in Northeast Brazil in May 2015. Since then, 35 countries and territories in the Americas have reported autochthonous transmission of ZIKV. In Nicaragua, the first case was reported in January 2016, and by April 2016, 130 cases had been identified by the national surveillance of the Ministry of Health. We are currently analyzing the epidemiology and clinical presentation of Zika in a prospective, community-based pediatric cohort study of dengue and chikungunya in Managua, Nicaragua. This study, ongoing since 2004, follows ~3,500 children aged 2-14 in a low- to middle-income area of Managua. Suspected Zika, chikungunya, and/or dengue cases and cases with undifferentiated fever are screened for ZIKV infection. Blood, saliva and urine samples are collected from suspected cases during the acute phase and at convalescence. Screening is conducted by real-time RT-PCR using the CDC protocol and a triplex ZIKV-CHIKV-DENV (ZCD) assay developed at Stanford University and validated in Nicaragua. From January to April 2016 (the dry season is March-May), 8 Zika cases were identified in the cohort (5 female and 3 male). Median age at presentation was 11 years (range: 7-14). One case required hospitalization. Six participants were DENV-naïve and two had had a previous DENV infection. As ZIKV transmission increases in the next rainy season, we will describe the natural history of ZIKV infection in our study populations and detection of ZIKV in blood, saliva and urine. In particular, clinical follow-up will continue after the acute phase, and participants requiring hospitalization and/or neurological examination will be transferred to our study hospital. We will also study potential immune enhancement between DENV and ZIKV infections. In our cohort, we will be able to compare ZIKV infection outcomes in participants with no, one or >2 previous DENV infections. Finally, as current serological assays cannot easily differentiate DENV and ZIKV, in particular if the patient has had a previous flavivirus infection or vaccination, we are developing and assessing new serological methods.

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THE SEASONAL INFLUENCE OF CLIMATE AND ENVIRONMENT ON YELLOW FEVER TRANSMISSION ACROSS AFRICA

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Yellow fever (YF) is a vector-borne, viral haemorrhagic fever endemic to Africa and Latin America with 90% of the burden occurring in Africa, transmitted primarily by *Aedes* spp, with *Ae. aegypti* the main vector for urban YF. Mosquito life cycle and viral replication in the mosquito heavily depend on climate indicators, particularly temperature and rainfall. We aimed to assess whether seasonal variations in climatic factors are associated with the seasonality of YF reports. We constructed a temperature suitability index for YF transmission, capturing the temperature dependence of mosquito life cycle and viral replication within the mosquito. We then fitted a series of generalised linear models to a dataset of YF reports across Africa, taking into account location and seasonality of occurrence for seasonal models and using the temperature suitability index, rainfall and the Enhanced Vegetation Index (EVI) as covariates alongside further demographic indicators. Model fit was assessed by the Area Under the Curve (AUC), and models were ranked by Akaike's Information Criterion. The seasonal interaction between temperature suitability and rainfall explained the seasonal and geographical patterns of YF occurrence well (AUC = 0.84), although models also including EVI performed significantly better (AUC = 0.88, $p < 0.001$). Despite the lower performance of the interaction of temperature suitability and rainfall compared to the EVI as a predictor of YF reports, the former offers a mechanistic explanation for the spatio-temporal variability and therefore enhances our understanding of the factors influencing YF transmission intensity. The description of seasonality in the transmission of YF opens up the possibility for the development of "early-warning" systems for outbreaks, which could facilitate the allocation of resources and guide vector-control programmes particularly if the insights gained here are combined with seasonal weather forecast data.

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ZIKA VIRUS: KNOWLEDGE GAPS AND VACCINE RESEARCH AND DEVELOPMENT CHALLENGES

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The recent emergence of Zika virus as a cause of severe disease has mobilized public health agencies, as well as commercial organizations, to embark on efforts to develop new approaches for combating this infection. Zika, a mosquito-borne flavivirus, has only recently been associated with severe disease in humans, including a variety of neurological complications that include microcephaly, congenital cerebral embryopathy and Guillain-Barré syndrome associated with an unprecedented spread of the virus. These links prompted the World Health Organization to list Zika as a "Public Health Emergency of International Concern" and the US CDC has now declared that Zika is a cause of microcephaly and other severe fetal brain defects. As exemplified during the recent Ebola outbreak, international and regional collaborations will be required to advance our understanding of Zika and to accelerate vaccine research and development. Potentially significant challenges for vaccine R&D have been identified, including; unknown incidence rates for clinical complications; while neutralizing antibodies will likely mediate protection, the level required is not yet known; the complexities of symptomatic and asymptomatic infection leading to clinical trials needing to demonstrate efficacy against disease/viremia and possibly fetal transmission for registration, and the development of diagnostic assays that avoid cross-reactivity with other flaviviruses. Sanofi Pasteur has initiated vaccine research and development activities to rapidly proceed through preclinical assessment into clinical evaluation. Assay optimization, animal model development and process improvements, as well as further disease understanding through potential surveillance of subjects participating in the follow up of the dengue efficacy study in Latin America, constitute the current focus of our work. Sanofi Pasteur's experience in licensed flavivirus vaccines will be leveraged against Zika vaccine development. Available results and progress will be presented.

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CO-INFECTION STUDIES BETWEEN WEST NILE VIRUS AND CULEX FLAVIVIRUS DETERMINE AN INHIBITORY EFFECT ON WEST NILE VIRUS INFECTION

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Vector-borne diseases remain a major public health concern as new diseases are emerging and previously controlled diseases are now resurging. West Nile virus (WNV) is a mosquito-transmitted flavivirus. Since its introduction in North America in 1999, it was spread rapidly to Central and South America. In Argentina, WNV was isolated in 2006; however, evidence of circulation by neutralizing antibodies in birds was detected by 2004. Still, human cases are scarce; interactions between vector, virus, and environment could be playing a role to explain it.The high rate of prevalence of Culex flavivirus (CxFV), an insect-specific flavivirus, detected in Argentina in previous studies, determined the importance to develop co-infection studies with WNV. For that purpose, *in vitro* studies were conducted in order to assess the potential of CxFV for blocking WNV infection. Co-infection assays using WNV and CxFV with different multiplicity of infection (MOI) in C6/36 cells from *Aedes albopictus,* were performed during 7 days. WNV titers were analyzed by plaque titration on 12-well plates of Vero cells.Results indicated that concurrent infection with CxFV resulted in a significant reduction in virus production of WNV. An inhibition of 100 fold reduction for WNV in presence of CxFV was detected. Reduction was statistically significant at 1 days post infection (dpi) and at 7 dpi using MOI of 0.1 for both viruses. Interesting, when MOI of CxFV was 10 or 100 times higher than MOI of WNV, reduction was statistically significant for every dpi. These results are in concordance with previous studies from other authors; co-infection resulted in suppression at higher total MOI, as a result of competition since the degree of resource depletion increases with MOI. This highlights that CxFV could be interfering in transmission of other flaviviruses of medical importance such as WNV. These results could explain, in part, the low level of transmission of WNV in Argentina. However, it will be necessary *in vivo* assays in order to evaluate this hypothesis. The results that are presented remark the potential interaction between flaviviruses.

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WEST NILE VIRUS INFECTION IN HUMAN AND MOUSE CORNEA TISSUE

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The purpose of this study was to determine the *in vitro* and *ex vivo* susceptibility of human corneal cells to West Nile virus (WNV) infection and evaluate the ability of the virus to disseminate to the corneas of infected mice. Human corneal epithelial cells were challenged with WNV, incubated for 1 to 6 days, and tested for evidence of WNV infection. Viral RNA and antigen were detected at every time point and the virus reached a peak titer of 2.5 x 10^7 plaque-forming units per milliliter (pfu/ml) at 3 days post-inoculation (PI). Corneas procured from donors were incubated in culture dishes containing WNV for 1 to 5 days and tested for evidence of WNV. Viral RNA and antigen were detected and the virus reached a mean peak titer of 4.9 x 10^4 pfu/ml at 5 days PI. Mice were inoculated intraperitoneally with WNV, and their eyes harvested at 2, 5, and 8 days PI and tested for evidence of WNV. Viral RNA was detected in anterior segment tissues in 4 of 9 systemically infected mice as early as 2 days PI. We conclude that human corneal cells support WNV replication *in vitro* and *ex vivo*, and WNV may disseminate into the corneas of experimentally infected mice. These findings indicate that corneal transmission cannot be ruled out as a novel mode of human-to-human WNV transmission and additional experiments should be conducted to assess this risk further.

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INTEGRATING SATELLITE REMOTE SENSING AND MOSQUITO SURVEILLANCE TO PREDICT HUMAN ARBOVIRAL DISEASE

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Mosquito-borne diseases present both novel and persistent challenges to public health, and predicting human risk is a perpetual, difficult exercise in integrating various streams of data. While remotely sensed environmental data (e.g. temperature and moisture) are readily available and are relevant to mosquito ecology, they do not directly capture other essential factors such as the immunity status of reservoir hosts. Mosquito surveillance data (e.g. infection status) are better measures of these hidden aspects of the disease transmission cycle, but are less accessible and more difficult to incorporate into operational forecasts of human disease. We present a flexible framework for predicting a mosquito-borne disease in humans, combining large volumes of meteorological data and sparse measurements of mosquito infection status. The approach was applied to reported human West Nile virus (WNV) infections in South Dakota, USA, 2004-2016. Data from NASA's North American Land Data Assimilation System (NLDAS) characterized temperature, precipitation, etc. on a daily basis. Early-season mosquito infection status data, measured in relatively few of the state's counties, were used to estimate influences not perceptible remotely. A statistical model of human WNV based on a parsimonious set of covariates achieved excellent discriminatory and predictive power, and yielded a straightforward description of human risk. Namely, long-term, interannual trends in human disease were explained by early-season mosquito infection status, and short-term variations by temperature. Early-season predictions for 2015 and 2016 are compared to observations. Scenarios for 2017, conditioned on predictive climate models, are discussed.

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WEST NILE VIRUS SUSCEPTIBILITY OF AMERICAN SINGER CANARIES: A LABORATORY MODEL OF A HIGHLY SUSCEPTIBLE AVIAN SPECIES

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In North America, West Nile virus (WNV) was first detected in 1999 in the New York area, and has since spread across the continental U.S., southern Canada, Mexico, Central America, and South America. Significant mortality, caused by WNV, was recognized in corvids (ravens, crows, magpies, and jays), and population declines have been recorded in corvids, as well as other avian species. Experimental challenge of birds with WNV has demonstrated susceptibility ranging from low - no mortality following challenge and WNV viremia unlikely to infect feeding mosquitoes - to high -frequent mortality and viremia very likely to infect feeding mosquitoes. Using wild-caught birds the extreme of this range might be observed with doves and corvids, with house sparrows filling the mid-range of susceptibility. Using wild-caught birds to examine species susceptibility to WNV has several disadvantages including difficulty of capture, presence of potential co-infections, such as avian malaria, and the potential for stress hormone release due to confinement at a research facility. We examined the susceptibility of mature American singer canaries (*Serinus canaria*) for WNV and found the species to be highly susceptible to the virus. Birds were inoculated with 10^5, 10^2, and 10^1 plaque forming units of WNV and mortality was observed in all birds by 5 days post inoculation. Viremia was quantitated by referring RT-PCR Ct scores to a standard curve and was comparable to the level of viremia that developed in American crows (*Corvus brachyrhynchos*) experimentally infected with WNV. Using a plaque assay, WNV was quantitated in tissues

obtained from diseased birds that were euthanized and was similar to viral load reported in WNV challenged American crows. Histopathology revealed lesions in a variety of tissues with liver, spleen, and kidney most severely affected. Immunohistochemistry (IHC) staining was pronounced in spleen and kidney sections. Brain and heart tissue were unremarkable on histopathology, and IHC stained sections were negative. American singer canaries provide a useful laboratory model to study the effects of WNV on birds highly susceptible to the virus.

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POLYMORPHISMS AT THE NS3-249 LOCI ARE ASSOCIATED WITH ALTERED VECTOR COMPETENCE OF *CULEX PIPIENS* FOR LINEAGE 2 WEST NILE VIRUSES

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Lineage 2 (L2) West Nile viruses (WNV) have undergone recent geographic expansions, resulting in outbreaks in eastern and southern Europe. Most notably, L2 WNV outbreaks have occurred annually in Greece since 2010. Genetic analysis of mosquito pool isolates made annually from Greece since 2010 have demonstrated the presence of a conserved L2 genetic variant containing a proline at NS3-249. This same genetic mutation has previously been associated with increased viremia and mortality in American crows (*Corvus brachyrhynchos*) infected with lineage 1 WNV strains. In order to evaluate if NS3-249 polymorphisms can alter L2 vector competence phenotypes, three parental L2 isolates (with NS3-249H) were compared to a Greek isolate from 2010 (NS10; NS3-249P) in *Culex pipiens* and *Cx. quinquefasciatus* mosquitoes. A lower infection rate was observed for *Cx. pipiens* orally exposed to NS10 compared to the three parental L2 isolates (NS3-249H); however, a similar lower infection rate for NS10 wasn't observed in Cx. quinquefasciatus mosquitoes. NS10 also exhibited reduced dissemination and transmission rates compared to SA89, a South African L2 (NS3-249H). To assess whether the NS3-249 loci is a determinant of vector competence in *Cx. pipiens*, vector competence of SA89, NS10 and corresponding mutants generated containing reciprocal substitutions at the NS3-249 loci (His vs Pro) was assessed. A higher infection rate was observed with the NS10-His mutant compared to wild type NS10. No differences in rates of dissemination or transmission were observed. Reciprocally, no difference in infection rate was observed for the SA88-Pro mutant relative to WT SA89; however, dissemination and transmission were lower. Increased avian replication could offset the negative impact of the NS10 Pro mutation observed on mosquito infectivity. In contrast, a loss in dissemination and transmission efficiency observed for the SA89-Pro mutant likely precluded maintenance of this mutant. As such, these data indicate the potential role mosquitoes play in restricting the emergence potential of high avian fitness variant due to reciprocal fitness effect in mosquito vectors.

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EVALUATION OF DIFFERENT VIRAL ENRICHMENT METHODS FOR WEST NILE VIRUS RNA EXTRACTION IN BRAIN

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Physical virus enrichment is thought to increase sensitivity and enhance detection of viral diversity, but these techniques are limited using brain tissue. Physical virus enrichment is especially useful for deep sequencing investigations. The hypotheses that elimination of host brain RNA will enhance sensitivity of viral detection and render undetectable virus available to sequence was investigated. Specifically, since West Nile virus (WNV) causes grave clinical signs in the face of low to undetectable virus, the aim of this research was to develop repeatable methods for detecting and generating WNV sequences from archived brain tissues of horses in which virus was either not detected or in limited quantity. Different WNV viral RNA extraction methods and host RNA separation methods were investigated by conducting artificial inoculation of WNV into horse brain. Twenty-one horse brains were collected and small pieces of brain from each were inoculated with WNV NY99 strain (low passage to maintain diversity) and each of them underwent eight different RNA extraction protocols. The protocols utilized low-speed centrifugation, syringe filtration, and nuclease treatment with combinations of these. The WNV viral RNA was analyzed using real-time PCR targeting WNV Envelope (e) protein and equine G3PDH. Deep sequencing was also performed using Illumina® platform. Real-time PCR results showed that the more enrichment applied to a sample, the less viral and host RNA was obtained. Data obtained for sequencing showed that more enrichment treatments resulted in more reads. Although calculation of single nucleotide polymorphism and diversity is necessary for full analysis, these results demonstrate that additional steps utilized for extraction of brain do not enhance detection of WNV. Methods for phylogenetic and phylodynamic studies in RNA viruses need to be tissue specific.

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DEVELOPMENT OF A LIVE ATTENUATED WEST NILE VIRUS VACCINE

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West Nile Virus (WNV) is an emerging human neurotropic pathogen that targets the central nervous system (CNS). Recent work has shown that inserting a single copy of a target for brain-expressed microRNAs (miRNAs) in the 3' noncoding region (3'NCR) of the flavivirus genome abolished virus neurovirulence in the mature mouse CNS. Furthermore, studies showed that introducing mir-124a target sequence into a flavivirus genome completely abolished neuroinvasivness in immunodeficient mice. Here, we developed a chimeric WNV / Dengue 4 virus vaccine candidate containing mir-124a targets in the junction of E-NS1 genes and in 3' non-coding region. miRNA targeted WNV / Dengue 4 was attenuated in 3-day old Swiss mice infected IC and did not cause neurologic disease in type I interferon receptor deficient mice infected intraperitoneally with10^4 PFU. To determine if miRNA targeted WNV /DEN4 is immunogenic and protects against challenge with wild type WNV, we inoculated C3H mice intraperitoneally with 10^5 PFU. Virus induced a high level of serum WNV-specific neutralizing antibodies in each immunized animal 28 days following inoculation. Moreover, 100% of immunized mice survived the lethal WNV challenge. We conclude that our developed virus has potential to be used as a live attenuated vaccine candidate against WNV disease.

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CLINICAL FEATURES OF ADMITTED CHILDREN WITH LABORATORY-CONFIRMED EBOLA VIRUS INFECTION DURING THE 2014-2015 EBOLA OUTBREAK IN GUINEA

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Background:Ebola outbreak in 2014 in West Africa is the largest in history and children are the most vulnerable. In this study, we aimed to understand the clinical characteristics of admitted children with confirmed Ebola virus infection. Methods: We have analyzed the data from 30447suspected or probable cases were admitted in ETC in Guinea. Among them 3062 were EVD confirmed between January 2014 and May 2015. Results:9155 were children less than 16 years and 518 of them were confirmed for EVD in Guinea. We observed that the main symptoms

were fever (92%), fatigue (85.7%)and anorexia (75.3%). The lethality rate were 82.9% and 55.8% for less 5 and 6-16 age groups respectively. age les than 5 and hospitalization less than 7 days were associated with death. Conclusion: We observed that the risk of getting the Ebola disease was significantly lower in children under 5 years. In contrast, the fatality rate was a higher rate in the same group (82.9 %).

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HEPATITIS E SEROPREVALENCE AMONG BLOOD DONORS IN RWANDA

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Hepatitis E Virus is mainly transmitted by the fecal-oral route but other routes have been reported. It usually causes self limited disease but can cause fulminant hepatitis in pregnant women. The prevalence of hepatitis E viral infection among Rwandan adults has not been reported previously. We aimed to determine the seroprevalence of Hepatitis E Virus among adult Rwandans, its relationship with pork consumption and other risk factors. A cross-sectional survey was conducted between November and December, 2014 on 309 blood donors in Rwanda. A subsequent nested case-control study assessed exposures in seropositive cases and seronegative controls. Hepatitis E Virus testing was performed by detection of anti- Hepatitis E Virus IgG antibodies. Demographic data and information about risk factors for Hepatitis E Virus infection were recorded. The average age was 30.6 years with a male to female ratio of 4:1. 54% were farmers .The overall Hepatitis E Virus seroprevalence was 13.3%. Rates of anti- Hepatitis E Virus positivity were lower in the Eastern Province and Kigali city than the Southern Province (p=0.01 and p=0.003, respectively). An association was found between pork consumption and Hepatitis E Virus seropositivity (p=0.04).The rate of pork consumption positively correlated with the rate of anti-HEV seropositivity (p=0.01). The analysis did not show an association between Hepatitis E Virus seropositivity and the source of drinking water, status of drinking water , exposure to animals or exposure activity. Therefore Hepatitis E Virus seroprevalence among blood donors in Rwanda is high. Anti- Hepatitis E Virus IgG seropositivity is likely associated with pork consumption and may be more prevalent in some regions of Rwanda.

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SINU VIRUS, A NOVEL ORTHOMYXOVIRUS RELATED TO MEMBERS OF THE THOGOTOVIRUS GENUS, ISOLATED FROM MOSQUITOES IN COLOMBIA

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During the past decade, many novel insect-specific viruses have been isolated from characterized in mosquitoes and phlebotomine sandflies. These insect-specific viruses are widely distributed geographically and represent a number of different virus taxa, including Togaviridae, Flaviviridae, Bunyaviridae, Rhabdoviridae, Mesoniviridae, Reoviridae and Birnaviridae. Here we report a novel insect-specific orthomyxovirus, designated CoB 38d, isolated in C6/36 cells from mosquitoes collected northwestern Colombia. Genome sequencing of CoB38d revealed the presence of a hexa-segmented RNA virus (Segments 1 to 6). Genetic analysis of each RNA segment demonstrated the presence of six distinct ORFs encoding for the following genes: PBS (Segment 1), PB1, (Segment 2), PA subunit (Segment 3), envelope glycoprotein gene (Segment 4), Nucleoprotein (Segment 5), and Membrane gene (Segment 6). Multiple sequence alignment, using all RNA segments of CoB 38d, revealed low nucleotide and amino acid identity (<50%) with all other members of the Orthomyxoviridae family. Phylogenetic analysis using the polymerase subunit 1 (PB1) amino acid sequences showed that the isolate is most closely related to members of the Thogotovirus genus. Based on the geographic origin of the isolate and phylogenetic analyses, we propose the name of Sinu virus (SINUV) and show that it is a new member of the family Orthomyxoviridae, and possibly a new genus within the family.

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PREDICTING THE GEOGRAPHIC SPREAD OF THE 2014-2016 WEST AFRICA EBOLA VIRUS DISEASE OUTBREAK

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Between 2013 and 2016 West Africa experienced the most geographically extensive outbreak of Ebola virus disease (EVD) recorded to date. It affected all districts in Sierra Leone and Liberia and most of Guinea, resulting in more than 11,301 deaths among 28,603 reported cases. The outbreak spread from its origin in Meliandou, Guinea by movements of infected individuals across the region. Understanding how human mobility influenced the transmission dynamics of this epidemic is important in planning responses to future outbreaks. Using empirical data on human mobility, we model the effect of human movement on the geographic diffusion of the outbreak, as well as on the dynamics of the growth and decline phases of the epidemic within and between each country's districts, to identify areas that were the main exporters and importers of disease transmission. We identify considerable spatio-temporal heterogeneity in transmission that is driven by human mobility both locally and between regions. We show that by incorporating a range of different human mobility models we improve predictions of both spatial spread and the prediction of the epidemic's trajectory. These results provide a robust approach to predicting the geographic spread of future outbreaks. Such models are crucial information for surveillance and control strategies both in preparation for, and in response to, future contagious disease outbreaks.

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SEVEN YEARS OF INFLUENZA SURVEILLANCE IN PRAMONGKUTKLAO HOSPITAL, THAILAND FROM 2009 TO 2015

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Influenza poses a constant threat to military forces around the world due to close living quarters and other environmental factors. The Virology department, AFRIMS conducted flu surveillance at Pramongkutklao Hospital, Thailand in order to assess this threat. Patients who presented with ILI were considered for enrollment. Two respiratory specimens were collected from each consented patient, a nasal swab for performing rapid testing and throat swab for RT-PCR testing / virus isolation. From 2009 to 2015, a total of 6263 patients with ILI were enrolled. A total of 4526 patients were negative and 1736 patients were found to be positive for Flu by rapid testing. Through RT-PCR testing, 2045 (32.6%) of the specimens were found to be positive for Flu. Of the positives, 1309 (64%) tested positive for Flu A (H1, H3) while 735 (36%) tested positive for Flu B viruses. Of Flu A subtypes, A/H1N1pdm contributed 577 (28.2%) of all Flu A positives while A/H3 accounted for 732 (35.8%). A subset of RT-PCR positive specimens were tested by virus isolation and sub-typed by HAI. From 2045 specimens cultured 779 (38%) yielded virus and 139 (6.8%) specimens were positive for A/H1N1pdm, 223 (11%) specimens were positive for A/H3, 216 (10.5%) specimens were positive for B/Victoria Lineage and 174 (8.5%) were positive for B/Yamagata Lineage. There are 26 (1.3%) Flu A specimens that could not be subtyped. All HAI sub-typing results correlate with RT-PCR results. For the entire surveillance period, specimens positive for A/H1N1pdm peaked in 2010, accounting for 307 (15%) of the specimens evaluated. Specimens positive for A/H3 were most commonly found in 2015, 222 (10.8%) and those positive for Flu B were very common in 2010 and 2014, accounting for (9.8% and 9.3%) of evaluated specimens, respectively. It was found that some of patients testing positive for influenza by RT-PCR in each year had received the seasonal Flu vaccine (within the last year). The vaccine efficacy was between 70% and 86%, except in 2014, where the level of protection was 28%. Most of the patients who received Flu vaccination one year prior to 2014 acquired Flu B, with incidence as high as 44.5%.

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PERSISTENCE OF IMMUNE RESPONSE IN EBOLA ZAIRE SURVIVORS FORTY YEARS AFTER INFECTION

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Ebola virus (EBOV) is a zoonotic filovirus that can produce highly lethal disease in humans. Presentation is non-specific, characterized by vomiting, diarrhea, fever and bleeding. The first reported outbreak of Ebola Zaire (ZEBOV) occurred in 1976 in the northwestern part of the Democratic Republic of Congo in the Yambuku village (DRC, former Zaire). While there is no licensed EBOV targeted vaccine or treatment, there is a growing body of evidence that offers hope for finding ways to pharmacologically mimic or boost the natural resistance some individuals have to EBOV. To better understand long-term humoral immunity following EBOV infection, we obtained blood from 12 remaining survivors from the Yambuku outbreak to determine if antibodies to ZEBOV were retained 40 years post initial infection. Serum samples from survivors were screened and analyzed using Human Anti-Zaire Ebola Virus Glycoprotein (GP) IgG ELISA Assay kits (Alpha diagnostic International, Inc) in Kinshasa, DRC. Manufacturer procedures were followed for all incubation and washing steps. Cutoffs were calculated using the provided calibrator 1 and subtracting the background optical density (OD). OD values at 450nm (OD450) were recorded. Results demonstrate that samples obtained 40 years post infection contain virus specific antibodies. We identified 5 positive subjects (OD ≥ Cal 2.5OD), 5 weak positives subject (≥Cal 1.0 OD and <Cal 2.5 OD) and two negative subject (<Cal 1 OD). These results provide insight into the duration of humoral immune response against ZEBOV and open the door to in depth characterization of the immune mechanism against EBOV as well as further studies are needed to provide more details on long term sequelae of the Ebola virus disease.

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ASSESSMENT OF MISEQ NEXT GENERATION SEQUENCING PROCEDURE FOR VIRAL PATHOGEN DETECTION IN SOUTHEAST ASIA

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Southeast Asian (SEA) countries have recently reported an increase in the detection and spread of emerging diseases. Currently, Next Generation Sequencing (NGS) is becoming an important tool for detection and analysis of emerging diseases. NGS is a reliable and accurate method of detection for emerging viral pathogens and provides critical information during outbreaks and routine disease surveillance. The Department of Virology, AFRIMS, has developed standard operating procedures and viral panels for performing NGS to assess the ability of laboratories to achieve reliable, accurate and rapid turnaround times for the detection of viral pathogens. The viral panels consist of the following commonly found viral pathogens circulating in Southeast Asia: Zika virus (ZIKV), dengue viruses (DENV) serotype 1-4, influenza A/pdmH1N and A/H3N2, and influenza B Victoria and Yamagata. Laboratories were trained to follow the SOPs and given the viral panel to perform NGS sequencing and pathogen identification. The laboratories were unaware of the viruses included in the panel before performing sequencing and analysis. A total of four different laboratories from three different countries including Thailand (2 laboratories), Cambodia, and Philippines were trained and given viral panels for sequencing. The panels were tested by 21 laboratory personnel from the different laboratories using the MiSeq® (Illumina) sequencing platform. All 21 laboratory personnel correctly identified the viral pathogens represented in the panel. Suggesting results from different locations and personnel are highly reproducible after laboratory technicians are trained to follow NGS sequencing SOP.

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ACCEPTANCE OF THE EBOLA VIRUS VACCINE BY THE COMMUNITY IN GUINEA

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The rVSV-ZEBOV vaccine was tested as Prevention means to Ebola virus through a vaccine trial led by the WHO in the region of Lower Guinea between April and July, 2015. The preliminary results of this vaccine trial showed that the rVSV-ZEBOV vaccine was safe and effective in the prevention of the Ebola virus disease ten days after its administration. With the prospect of moving to scale up for a large part of the population, a study on acceptability of the vacccine was conducted to identify possible challenges. Interviews were conducted on the basis of a standard questionnaire administered to 209 people in Coyah, Forécariah, Kindia and Dubreka. Quantitative datas were entered in the Epidata version 3.1 software and then exported to the software Stata 13 (Stata Corp., Texas, USA) for analysis. Descriptive statistics and chi square test at 95% confidence interval were used to measure the association of selected variables in the study. Nearly 78% of the people interviewed agreed to be vaccinated and 73% of those interviewed were willing to encourage their close ones and relatives to take this vaccine. Nevertheless, 22% of the survey participants expressed doubts or had mixed feelings about the vaccine. The main concerns raised in the qualitative interviews conducted focused on the quality of the vaccine, the side effects of the vaccine, fear of being contaminated by Ebola during vaccination exercise and lack of adequate information. Knowledge of the existence of the new vaccine was higher in areas where the community was involved in response activities (Coyah and Forecariah) than in the areas where the community was not involved (Dubreka and Kindia). Similarly the willingness to be vaccinated (p= 0.003) or encourage relatives to do the same were higher in the prefectures of Forécariah and Coyah compared to the Prefectures Dubreka and Kindia (p = 0.011).The results of this study show that there is need for public information on the new Ebola vaccine, especially in prefectures where acceptance is low, by involving local authorities, community leaders and community-based organisations to conduct outreach sensitization outreaches.

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COMPARISON OF CULTURE, SINGLE AND MULTIPLEX REAL-TIME PCR FOR DETECTION OF SABIN POLIOVIRUS SHEDDING IN RECENTLY VACCINATED INDIAN CHILDREN

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Poliovirus identification in clinical and environmental samples is a crucial component of the global polio eradication program led by the World Health Organization (WHO). Although culture is considered the gold standard for poliovirus detection from stool samples, real-time polymerase chain reaction (PCR) has emerged as a faster and more sensitive alternative. In this study, we evaluated the performance of culture in the L20B cell line and two real-time PCR assays (for single poliovirus types and a one step multiplex real-time PCR) in the detection of Sabin poliovirus from stool samples of recently vaccinated Indian children. A random set of 80 stool samples from infants vaccinated with trivalent OPV (tOPV) at 6 weeks of age was selected for the study. The samples were collected as part of a clinical trial (CTRI/2012/05/002677) evaluating supplementation with zinc and/or probiotics to enhance the immune response of oral rotavirus vaccine and tOPV in Indian infants which was conducted at Vellore, India, between July 2012, and February 2013. Of the 80 stool samples tested, 55 (68.75%) were positive by culture. In contrast, 61 (76.25%) and 60 (75%) samples were positive for poliovirus by the single and one step multiplex real-time PCR assays respectively. If culture in L20B cell line is considered the gold standard for poliovirus detection, the sensitivity of singleplex and multiplex real-time PCR were 94.5% and 92.7% respectively, while the specificity was 64% for both the PCR assays. If the PCR methods are considered the gold standard for detection, the sensitivity of culture was 85% and the specificity varied from 84.2% to 80%. The quantity of virus as estimated by Ct values differed between culture positive and negative samples. Culture positive samples had significantly lower Ct values than samples that were negative in culture, ($p<0.05$), except for Sabin 3 detection by multiplex real-time PCR where the difference did not reach statistical significance. To conclude, the two real-time PCR assays for detection of single or multiple Sabin polioviruses in stool samples from vaccinated children were more sensitive than culture.

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THE PREVALENCE OF HUMAN PAPILLOMAVIRUS (HPV) GENOTYPES AMONG WOMEN WHO PRESENTED FOR ROUTINE PAP SMEAR TEST IN THE UNITED ARAB EMIRATES

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Cervical cancer is the second most common cancer that affects women worldwide. Some genotypes of human papillomaviruses cause genital infection and can lead to cervical cancer. Therefore, the aim of this study was to determine the prevalence of HPV genotypes among women who presented themselves for a routine Pap smear test in the United Arab Emirates. A total of one hundred three (n=103) samples were collected between April-October 2011 and January-October 2012. Pap smear method was used to detect the abnormal cytology results of HPV. Then, initial test which is Digene hc2 was used to detect high/low risk HPV and DNA was extracted from these samples .After that, PCR was used to amplify the desired gene of HPV and the specific genotypes of HPVs were identified by an automated sequencing. One patient was excluded from the study because of incomplete data and a total of 102 were used for the final analysis. Based on the collected data, 39 (38.2%) samples out of 102 samples were positive for HPV DNA and 63 (61.8%) samples had negative results for HPV DNA. Meanwhile twenty seven (26.5%) samples were detected positive for HPV with PCR as well as Pap smear method. However, twenty (42.6%) samples were identifies positive for HPV with Pap smear method; were negative for HPV DNA by PCR. Among 102 samples; HPV types were identified as following; 4.9% samples with HPV-16, 3.9% samples with HPV-6 and 3.9% samples with HPV-53. The result illustrated that the most prevalent high risk HPV type among women in the United Arab Emirates was HPV-16. In conclusion, the high prevalence of carcinogenic HPV in our study, screening the normal population of women in the United Arab Emirates is recommended by both Pap smear and HPV DNA tests to avoid any false positive or negative results. The genotyping is essential for the decisions by the national government to support vaccination initiatives.

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INFLUENZA, DENGUE, CHIKUNGUNYA AND MULTI-DRUG RESISTANT BACTERIA SURVEILLANCE IN A PHILIPPINE TERTIARY HOSPITAL

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USAMD-AFRIMS has collaborated with the V Luna General Hospital since 2008 in emerging and re-emerging disease surveillance including antimicrobial resistance. Influenza and dengue virus was identified by real-time and conventional Polymerase Chain Reaction (PCR) respectively. Bacterial identification and antibiotic susceptibility testing was conducted using the Microscan Walkaway 40 Plus System. Mycobacterium tuberculosis was identified using the GeneXpert (Cepheid). From Feb 2008 - Mar 2016, 950/2,896 (33%) of collected respiratory specimens were positive for influenza: 307 FluA/H3; 325 Flu A/pdmH1; 289 Flu B; 11 Flu A/H1. 224/552 (44%) specimens collected from Nov 2012 - Feb 2016 were positive for dengue virus with the following serotypes detected: 69 DENV-1, 71 DENV-2, 61 DENV-3 and 41 DENV-4. 11/112 (10%) of clinically diagnosed dengue cases were confirmed to be chikungunya after PCR testing for both dengue virus and chikungunya virus. From Aug 2013 - Dec 2015, 28.3% (45/159) of Klebsiella spp. isolates and 2.7% (4/146) of *Escherichia coli* isolates were identified as imipenem resistant. 39.5% (30/76) of Acinetobacter spp. and 19.5% (30/154) of Pseudomonas aeruginosa isolates were resistant to all antibiotics in the Microscan negative breakpoint combo 30 and 34 panels. 70.4% (95/135) of Staphylococcus aureus isolates were methicillin resistant. 22% (18/81) sputum samples collected from Mar 2015 - Feb 2016 were identified as Mycobacterium tuberculosis and 22% (4/18) of the M. tuberculosis isolates were rifampicin resistant. This paper describes a research collaboration focusing on monitoring for emerging & re-emerging diseases in a tertiary military hospital setting. This information is crucial to define disease burden as well as to strengthen disease surveillance to better characterize and improve early detection and containment strategies.

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SEROPREVALENCE OF EBOLA VIRUS AMONG HEALTH CARE WORKERS IN THE TSHUAPA DISTRICT, DEMOCRATIC REPUBLIC OF CONGO

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Ebola virus disease (EVD) is caused by a zoonotic filovirus infection that can be highly lethal in humans. Since 1976, a total of seven confirmed EVD outbreaks have occurred in the Democratic Republic of Congo (DRC). Health care workers (HCW) are at particularly high risk of EVD infection given the high titers of virus in bodily fluids and lack of compliance with precautions to prevent exposure either due to lack of knowledge, training or equipment. Therefore, we conducted a serosurvey among HCWs attending a workshop in the Tshuapa district's capital, Boende, DRC. Field collection occurred in September 2015. Interviews and blood specimens were collected from all consenting individuals. Serum samples from 70 HCWs based in12 health zones in Tshuapa were screened for Ebola virus zaire (ZEBOV) GP Ig detection using Human Anti-Zaire Ebola Virus Glycoprotein (GP) IgG ELISA Assay kits (Alpha diagnostic International, Inc.) in Kinshasa, DRC. Among the 70 health care workers, 43% (n=30) were seropositive for ZEBOV GP IgG, 49% (n=40) were seronegative, and 21% (n=17) were indeterminate. Among those seropositive, 68% (n=34) had participated in 1 or more EVD outbreaks of which 41% (n=14) had used Personal Protective Equipment (PPE). Among all HCWs, only 10% (n=6) suspected that they had become infected with EVD, however none were confirmed through testing. Our findings suggest that some Tshuapa HCW's may be highly exposed to EBOV. While our estimate of seroprevalence is higher than other studies, this may be explained by HCW participation in the 2014 Boende outbreak or by asymptomatic exposure in an endemic region. Regardless, increased biosafety training is needed to prevent transmission in HCWs.

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SUSCEPTIBILITY OF BAT CELL LINES R06E AND R05T DERIVED FROM THE EGYPTIAN FRUIT BAT TO PATHOGENIC HUMAN VIRUSES

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Recent surveys have found that bats harbor viruses from at least 24 viral families and are suspected reservoirs for many emerging human pathogenic viruses. While the variety of viruses identified in bats is staggering, many have been detected only by serology or molecular techniques, with the isolation of virus particles being a challenge in existing cell lines. Dr. Ingo Jordan's group at ProBioGen, Germany developed the R06E and R05T primary cell lines as potential tools for the propagation of bat viruses. These cultures are adenovirus E1 immortalized cell lines derived from Rousettus aegyptiacus fruit bat fetal cells. Both cell lines have been verified by BEI Resources to be of R. aegyptiacus origin through cytochrome c oxidase subunit 1 (CO1) barcoding. Select viruses from BEI Resources were passaged in R06E and R05T to evaluate infectivity and the initial host cell line used for the propagation of the stock virus was run as a control for optimal growth. The tissue culture infectious dose (TCID50) was determined for each passage and cytopathic effect was documented. The first two viruses tested were the Sindbis (Togaviridae) and Tacaribe (Arenaviridae) viruses, which are known to be found in bats. Both bat cell lines demonstrated cytopathic effects comparable to those observed in Vero E6 control cell line. Susceptibility to a particular virus was observed in cells that did not exhibit a significant decrease in viral titer after three passages. Testing is underway for cell line susceptibility to additional virus families, including Coronaviridae (Middle East respiratory syndrome virus) and Flaviviridae (Zika virus). The verified pure culture R06E and R05T bat cell lines support the growth of zoonotic viruses detected in both humans and bats, and can be used to study crossover between human and bat pathogens. Future studies are required to demonstrate whether these cells support the isolation and propagation of bat viruses that are currently detected by molecular methods only. These cell lines should support further research in bat immunology and virus-host interactions and are available through NIAID's BEI Resources.

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ETIOLOGY OF ACUTE FEBRILE ILLNESSES AMONG PREGNANT WOMEN FROM THE SENTINEL ENHANCED DENGUE SURVEILLANCE SYSTEM (SEDSS) IN PUERTO RICO

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Acute febrile illnesses (AFI), characterized by undifferentiated fever, present a risk for pregnant women due the level of suppression of the immunological system during pregnancy. Fever during pregnancy has been consistently associated with congenital anomalies, including neural tube defects, craniofacial malformations, and cardiac anomalies, with 1.5-3 fold increased risk after febrile illness in the first trimester. There is no published evidence that describes the etiology of AFI during pregnancy in Puerto Rico, an island with endemic transmission of arboviruses such as dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV) viruses. Our study aims to describe the infectious agents and outcomes of AFI during pregnancy by trimester of participants from the Sentinel Enhanced Dengue Surveillance System (SEDSS), a facility-based epidemiologic platform established in southern PR. Pregnant women with fever of <7 days were enrolled from May 7, 2012 to May 6, 2015. Clinical data was collected and specimens were obtained and tested by RT-PCR and immunodiagnostic methods as appropriate for DENV-1-4, CHIKV, Leptospira species, enteroviruses, influenza A/B viruses, and other respiratory viruses. A total of 152 pregnant women were enrolled in SEDSS, half (46%) of which were aged 20-24 years. A pathogen was identified in 93 (61%) cases: 56 (37%) had CHIKV, 23 (15%) influenza A/B virus, 6 (4%) DENV, 5 (3%) other respiratory virus, 2 (1%) enterovirus and 1 (1%) co-infected. When analyzing by trimester of pregnancy, one (1.1%) DENV and 2 (2.2%) CHIKV cases were found during the first trimester; 3 (3.2%) DENV and 25 (26.9%) CHIKV during the second; and 2 (2.2%) dengue and 26 (28%) CHIKV during the third. All DENV cases were diagnosed between 2012 (n=4) and 2013 (n=2) and all CHIKV cases between 2014 (n=53) and 2015 (n=3). In conclusion, the surveillance system was able to identify pathogens associated to AFI during pregnancy. The most recent CHIKV outbreak contributed the majority of the cases presenting in pregnant women. Of interest are the outcomes of the mother and newborns, for which analysis is currently being done and will be presented.

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LEISHMANIA INFECTION IN SANDFLIES IN A CUTANEOUS LEISHMANIASIS FOCUS IN GHANA

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The distribution and *Leishmania* infection in sandflies was examined in the endemic cutaneous leishmaniasis (CL) communities of the Volta region, Ghana. CL was first report in 1999 by the Ghana Health Service in the Ho, Hohoe and kpando municipality. Since the first outbreak of the disease, there have be increasing reports of the disease in various villages in the Volta Region of Ghana. In this study, to identify natural infection by *Leishmania* sp. in insect vectors of CL, entomological survey was performed in three endemic communities (Dodome Awiasu, Dodome dogblome and Lume atsyame) in the Volta region. From October 2012 to February 2013, a total of 4219 female sandflies were captured with CDC light traps and dissected for morphological identification. It was observed the 20(0.5%) female sandflies were identified from the genus *Phlebotomus* and 4199 (99.5%) from the *Sergentomyia*. To determine leismania infection in female sandlies, DNA was extracted from pools of sandfly species ranging from 1 to 25 dissected females. In considering the pools of individual sandfly species, *Leishmania* sp. infection of 0.0384% was detected in a pool of 7 (5.7%) *S. africana* female sandflies out of 122 pools using PCR. This is the first report of natural infection by *Leishmania* sp. in *S. africana* in Ghana. This observation that *S. africana* are naturally infected by *Leishmania* sp., suggest that the sandfly species might play a role in the transmission of cutaneous leishmaniasis within the Volta Region of Ghana.

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MEGALOPYGE OPERCULARIS: THE STING THAT KEEPS ON STINGING, 1798-2016

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Megalopyge opercularis (Order: Lepidoptera) is known commonly by a number of monikers, most notably the "wooly asp" and the "puss caterpillar." First described by the pioneering English lepidopterist, James Edward Smith in 1798, the Megalopyge opercularis quickly gained a reputation for its painful "urticating sting." In the more than two centuries that have passed since Smith first described the Megalopyge opercularis, a steady flow of studies has made it one of the most widely studied of the "urticating lepidoptera." This presentation will examine reports on Megalopyge opercularis since the late 18th century. Particular attention will be paid to their place within the historical development of medical entomology.

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ECTOPARASITE INFESTATION OF A HOSPITAL DUE TO NESTING CLIFF SWALLOWS

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The Cliff Swallow (*Petrochelidon pyrrhonota*) is a migratory passerine that constructs conical mud nests in colonies during the summer throughout North America. A large colony of swallows was established in the window eaves of a hospital. In July of 2015 the Infection Prevention Committee was informed of unusual "bugs" found in patient rooms. Two avian ectoparasites were identified, *Argas cooleyi* (argasid tick) and *Oeciacus vicarius* (swallow bug). Initial surveillance revealed ectoparasites in twenty-two locations (hallways, entrance points, and patient rooms) correlating with the presence of swallow nesting sites. Eradication of the ectoparasites was executed over a six-month period which included insecticide application and re-caulking of window sills throughout the structure. However, the swallows were not disturbed during their nesting period as required by the Migratory Bird Act and by late November of 2015 the birds had migrated. Removal of the abandoned nests (n=267) soon followed. Installation of bird netting in locations of prior and/or potential nesting sites completed the project. Since the discovery of this infestation, bimonthly surveillance took place to assess the ectoparasite burden. Decreasing numbers were recorded with each month and by the end of December 2015 no more ectoparasites were discovered. Argasid ticks are known to opportunistically feed on humans in the right circumstances. The collected ticks (n=237) were tested for the presence of human blood by an immunohistochemical test specific for human glycophorin A (surface protein on red blood cells). The results indicated that human blood was present in some of the collected ticks. The nesting of Cliff Swallows on man-made structures, including medical facilities, may

facilitate an accompanying ectoparasite infestation as demonstrated in this case. Argasid ticks are known vectors worldwide in the transmission of arboviruses and bacterial (*Borrelia* and *Rickettsia* species) pathogens that can infect both animal and human hosts. This unusual ectoparasite infestation involved two arthropods whose domiciles needed to be removed in order to eradicate them.

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FIRST DETECTION OF *LEISHMANIA TROPICA* IN NATURALLY INFECTED *PHLEBOTOMUS PAPATASI* (DIPTERA: PSYCHODIDAE) IN NORTH SINAI, EGYPT

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Cutaneous Leishmaniasis (CL) is endemic in the Middle East and prevalent in the Sinai Peninsula, Egypt. Cutaneous Leishmaniasis is caused by *Leishmania major* or *L. tropica. Leishmania major* is transmitted by a bite of female *Phlebotomus papatasi* (*P. papatasi*), the principal sand fly vector throughout the Mediterranean basin, Middle East, Central Asia, and East Africa. *Leishmania tropica* occurs widely in Israel, Jordan Valley, and the Negev Desert. Personnel participating in the peace keeping in the Sinai-based Multinational Force and Observers (MFO) camps, are at risk to sand fly bites. Herein we describe surveillance of *Leishmania* disease vectors in the Sinai. Vector surveillance was conducted at multiple MFO remote sites using the Centers for Disease Control and Prevention (CDC) light traps. Species identification was done based on the morphological characters. *Leishmania* species was detected by amplification of the small-subunit ribosomal RNA (ssu rRNA) gene. *Phlebotomus papatasi* (Scopoli) comprised 999 (97%) of 1,030 collected sand flies. Out of 354 tested *P. papatasi* females, 106 (29.9%) of 354 were positive for *Leishmania* ssu rRNA gene by Real-time PCR. Restriction fragment length polymorphism (RFLP) typing of the internal transcribed region (ITS1) of ssu rRNA amplicons from positive samples confirmed *L. tropica* and *L. major* fingerprints. Twenty of 106 (19%) females captured were infected with *L. tropica,* while 86 (81%) females were infected with *L. major.* This study reports the first detection of *L. tropica* in *P. papatasi* from Sinai, Egypt. The abundance of infected and non-gravid *P. papatasi* suggests a vectorial capacity of this species to transmit *L. tropica*. This work emphasizes the value of systematic survey of *Leishmania* reservoir hosts and vectors.

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DEVELOPMENT OF A MULTIPLEX REAL-TIME PCR ASSAY FOR IDENTIFICATION OF *PHLEBOTOMUS* SAND FLY SPECIES INVOLVED IN *LEISHMANIA* TRANSMISSION

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Phlebotomine sand flies (Diptera, Psychodidae, Phlebotominae) are the principal vectors of *Leishmania* parasites. Phlebotomine vectors are significant threats to public health in areas such as North Africa, the Mediterranean basin and the Middle East. Of 800 sand fly species published, 10% are competent vectors of Leishmaniasis. Identification of sand flies in *Leishmania*-endemic areas is paramount. Morphological sand fly identification, a process reliant on discernment of delicate features, is time consuming and requires taxonomic expertise. Molecular-based identification methods provide an alternative approach to morphological identification; however few studies have developed PCR techniques to discriminate between specific sand fly species. Here we describe the development of a multiplex TaqMan real-time PCR assay for the detection of three *Leishmania* vector species (*Phlebotomus papatasi*, *Ph. sergenti*, and *Ph. alexandri*) in a single reaction. DNA was extracted from individual *Phlebotomus* sandfly species that have been maintained in continuous laboratory colonies. We design three species-specific primers and TaqMan probes targeting a consensus region of 18S ribosomal DNA gene using sequences retrieved from the GenBank in addition to other in-house sequences generated from individual *Phlebotomus* specimens (target species) and *Sergentomyia* specimens (non-target species). To evaluate the sensitivity of the assay serial dilutions of the three target species in mixed-pools were prepared at ratios ranging from 1:10 to 1:50. Mixed-pools of target to non-target species were used to determine the specificity of the assay. This TaqMan multiplex real-time PCR method identified *Ph. papatasi*, *Ph. sergenti*, and *Ph. alexandri* effectively, and demonstrates a higher sensitivity up to a dilution of 1:20. Mixed pools of target and non-target species showed the assay to be 100% specific. The present study provides a quick and reliable one-step multiplex assay for the identification of *Leishmania* vectors in field-caught sand flies where pools of vector and non-vector species share the same ecological niche.

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IMMUNE MODULATING MOLECULES OF *SARCOPTES SCABIEI*

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The ectoparasitic mite *Sarcoptes scabiei* uses a variety of mechanisms to evade its host allowing a parasitic colony to become established. One of these strategies involves modulation of the innate and adaptive immune responses in the host skin. Having previously sequenced the scabies mite genome and characterized > 200 of the proteins produced by this mite, we are now seeking to identify the molecules that this mite uses to immunomodulate the function of fibroblasts and endothelial cells of the microvasculature of the host dermis. Several proteins were selected for study and their genes were chemically synthesized and cloned into appropriate vectors for expression in *Escherichia coli.* Fusion proteins were purified and used to challenge normal human dermal fibroblasts (NHDF) and microvascular endothelial cells (HMVEC). Two of these clones stimulated the secretion of IL-6, IL-8, and GCSF by NHDF and of IL-6 and GM-CSF by HMVEC in a dose dependent manner. These responses are similar to the cellular responses to whole scabies mite extract suggesting that these molecules may be among those used by the mite to protect itself from the host. Neither protein bound antibody from the serum of any scabies infested hosts tested suggesting that they are able to evade the host's adaptive immune system as well.

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CONCEALED ANTIGENS AS VACCINE TARGETS FOR CONTROLLING TRIATOMINES, VECTORS OF CHAGAS DISEASE

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Controlling triatomine (Hemiptera:Reduviidae) bugs is an important aspect of managing the spread of *Trypanosoma cruzi*, the causative agent of Chagas disease, for which no vaccine is currently available. Triatomine control methods depend on insecticide use and environmental management. Insecticide resistance and the ecology of some triatomine species, that tend to persistently re-invade domestic and peridomestic structures following the application of these control methods, may require additional control approaches. The development of a triatomine vaccine to target the insect vector would provide an additional prevention and control method. Vaccination of animal hosts with triatomine antigens would cause the hosts' immune system to produce antibodies that would be ingested in a blood meal. Many proteins within the triatomine salivary glands and midgut are critical to the acquisition and digestion of obligatory blood meals. Thus, inhibiting one or more of these proteins using ingested antibodies could interrupt important biological processes required for triatomine survival. To this end, the selection, recombinant expression and

vaccine evaluation of twelve Rhodnius prolixus (Hemiptera:Reduviidae) midgut protein targets are discussed as well as the concept of "exposed" and "concealed" antigens.

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OVIPOSITION ATTRACTANTS OF *PHLEBOTOMUS PAPATASI* - PRELIMINARY RESULTS

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Phlebotomine sand flies transmit protozoan parasites (*Leishmania* spp.), bacterial (*Bartonella bacilliformis*), and viral pathogens. An alternative approach to the traditional delivery of an insecticide to the vector is to bring the vector to the insecticide using an attractant. In the context of controlling vector-borne disease, oviposition-site attractants are expected to be highly effective because they target gravid females that are responsible for transmission of the pathogen and amplifying vector populations. Decomposing organic matter is the main food source for sand fly larvae. We therefore hypothesize that gravid sand flies are differentially attracted in a dose-dependent manner to a blend of fecal- and microbially-derived chemical cues associated with the decomposition of fecal material, as well as to signals from eggs and larvae which indicate suitable oviposition sites. Our overall goal is to develop and optimize an attractive blend of semiochemicals that would function as a lure for oviposition-site seeking sand fly females using *Phlebotomus papatasi* (vector of old-world cutaneous leishmaniasis) as a model system. We will apply an integrated interdisciplinary approach including behavioral, electrophysiological, and microbiological studies to address the following specific aims: (1) Identify the most attractive and oviposition stimulating conspecific stages, rearing medium, and saprophytic microbes; (2) Isolate and identify oviposition attractants and stimulants from the most attractive conspecific stage, rearing medium, and microbial isolates; and (3) Develop an optimal blend of oviposition attractants and stimulants and evaluate it at the micro- and meso-scales. This proposed study introduces several novel and innovative approaches including: (1) Application of an integrated approach including behavioral, electrophysiological, analytical and microbiological investigations; (2) Study a neglected aspect of oviposition - the role of saprophytic fungi as indicators of suitable oviposition sites; (3) Evaluate the effectiveness of the optimized blends at the scale of meters using a wind-tunnel.

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RNASEQ OF WILD CAUGHT SAND FLY *PHLEBOTOMUS CHINENSIS* FROM SICHUAN, CHINA

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Sandfly *Phlebotomus chinensis* is responsible for the transmission of visceral leishmaniasis in China. In this study we conducted RNA-seq for head and body of both male and female sandflies to determine the transcriptional differences between females and males, head and body. Wild caught sandfly specimens were collected in an endemic region in Sichuan, China. cDNA libraries of male heads and male bodies, female heads and female bodies were prepared for RNAseq using Illumina paired-end sequencing technology. Approximately 128 million clean reads were assembled into 32,628 unigenes with an average length of 1,235 bp, an N50 of 2,551 bp, and an average GC content of 47.88%. All unigenes have good hits against Nr, 26,601 (46%) have Swiss-Prot hits, 5,244 (19.15%) have KEGG annotation. Furthermore, a total of 256,890 SNPs were identified. Transcriptomic comparisons between head vs. body as well as males vs. females revealed ~5,000 unigenes that were deferentially expressed between these samples. Our RNAseq data provide a comprehensive transcriptomic resource for Ph. chinensis, and will facilitate further studies on genetic and genomics. The genome of *Ph. chinensis* has been sequenced. Efforts are now underway to assemble and annotate the genome.

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TOXICITY OF PLANT EXTRACTS AND PYRETHROIDS TO CATTLE TICK, *BOOPHILUS* SPECIES (IXODIDA : IXODIDAE)

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Ticks transmit protozoan, bacterial, rickettsial and viral pathogens to both human and animals worldwide leading to enormous economic losses to the livestock industry. However, the indiscriminate use of synthetic acaricides for tick control has contributed to the development of resistance in tick population. There is an urgent need to search for effective and safe alternative means of control. A laboratory evaluation was therefore carried out to determine the toxicity of aqueous extracts of two plants (*Azadirachta indica* and *Annona muricata* leaf) and two synthetic pyretheroids (deltamethrin and lambda-cyhalothrin) to tick species of cattle (*Boophilus* species). Wild adult ticks collected from cattles around Amansea, Awka, Anambra state, Nigeria were exposed to the acaricides using adult immersion test. Five concentrations of the plant extracts (500, 250, 125, 62.5 and 31.2µL/mL) and synthetic pyrethroids (50, 25, 12.5, 6.25 and 3.12µg/mL) were used to immerse 10 active adult *Boophilus* ticks. Each concentration was done in duplicate and replicated thrice and a control was included. Mortality post exposure was monitored and recorded at 24, 48, 72 and 96 hours and data was analysed using log-probit regression analysis. Results showed that dose-related mortality responses were observed at different time intervals. Mortalities of 100% and 76.7% respectively was recorded for *A. indica* and *A. muricata* at 50% concentration while exposure to deltamethrin and lambda-cyhalothrin resulted in 86.7% and 76.6% mortality respectively at 50µg/mL concentration. LD_{50} values for adult *Boophilus* ticks were 5.36% and 14.40% for *A. indica* and A. *muricata* while that of deltamethrin and lambda-cyhalothrin were 6.00µg/ml and 2.25µg/ml respectively. The LT_{50} values were 63.66 and 119 hours for *A. indica* and *A. muricata* and 66.69 and 76.13 hours for deltamethrin and lambda-cyhalothrin respectively. The study suggests that botanical extracts could serve as a good alternative to synthetic acaricides.

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A BANDIT MODEL TO OPTIMIZE ENTOMOLOGICAL SURVEYS

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Although vector-control campaigns have prevented considerable morbidity and mortality from vector-borne diseases, there is increasingly a need to find strategies that accurately and efficiently identify foci of transmission based on limited information. Currently, control strategies rely on systematic screening for infestations and require time- and resource-intensive surveillance, while less resource-intensive reactive control strategies are often inadequate to detect the emergence or re-emergence of disease vectors. We propose and test a novel spatial search

strategy. Our strategy uses a multi-armed bandit algorithm to select among a number of proposed search areas each day based on results from previous days, and ultimately localizes searches to the most heavily infested areas. As an online optimization strategy, it obviates the need for preliminary surveys and responds to changing conditions experienced by the field teams. Furthermore, as a bandit strategy, the algorithm is designed to optimally balance between exploiting high-prevalence areas and exploring unknown areas that may have a high infestation burden. We investigate the properties of this strategy using simulation studies and apply it to retrospective data from several recent vector-control campaigns in Arequipa, Peru.

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VECTOR CONTROL USING LONG-LASTING INSECTICIDAL NETS AGAINST VISCERAL LEISHMANIASIS IN BANGLADESH

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Vector control takes on a role as important part in controlling the diseases transmitted by arthropods such as leishmaniasis. Visceral leishmaniasis (VL) is one of the major public health problem in Bangladesh, but the most appropriate vector control measures is still a matter of debate. Here, the efficacy of permethrin treated long-lasting insecticidal nets (LLINs), Olyset® and Olyset® Plus against field collected sand flies was evaluated in a VL endemic area in Bangladesh. Sand fly bioassays were conducted according to the WHO-approved cone test methodology with modification. A major species of tested sand flies (91.28%) was *Phlebotomus argentipes*. Sand flies were introduced into a plastic cone attached with a piece of Olyset® or Olyset® Plus for 3 min and mortality and knock down rate were continuously recorded until 24 hrs after the exposure. Approximately 20-25 sand flies were used in each set, and the tests were repeated 4 times. The mortality of sand flies recorded on 24 hrs was 100% in Olyset® Plus group while that mortality of sand flies in Olyset® group was 83.63% (corrected mortality = (% test mortality – % control mortality) / (100 – % control mortality) × 100). The knowledge, attitude and practice of people live in endemic area about VL are also essential in order to propose successful vector control strategies. Therefore, questionnaire-based survey was also demonstrated to know whether the vector control using LLINs is sustainable application for the people lived in endemic are or not. The questionnaire was consists of three sections, socio-demographic characteristics, knowledge of VL and history of VL, and perceptions of VL vector control. Based on the analysis of 1393 households, the knowledge, attitude and practice of people live in endemic area about VL were relatively low. Though utilization of LLINs is promising, its ownership is noticeably low. Vector control using permethrin treated LLINs can be one of a potential tool for reducing the morbidity rate of VL in endemic area in Bangladesh. This works was supported by Science and Technology Research Partnership for Sustainable Development.

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EXPERIMENTAL SUSCEPTIBILITY OF *LUTZOMYIA LONGIPALPIS* TO DIFFERENT SPECIES OF *LEISHMANIA*

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In order to better understand the epidemiology of leishmaniasis and the parasite transmission it is important to develop studies on the *Leishmania*-vector interaction. The knowledge of the process of the sand fly-parasite interaction can contribute for new control strategies of a disease that until now has no effective vaccine a and limited range of drugs for the treatment. In nature, some sandfly species show remarkable specificity to transmit an exclusive *Leishmania* species (non-permissive vector) while others can be vectors of more than one parasite species (permissive vector). However, in in the laboratory some sandfly like *Lutzomyia longipalpis*, exclusive vector of *L. chagasi infantum*, can be experimentally infected by different parasite species. Here, we analyzed in details the development of four *Leishmania* species: *Leishmania major, L. amazonensis, L. braziliensis* and *L. chagasi* in the *Lu. longipalpis*, a New World sandfly. Experimental infections were conducted using three parasite doses (4x107, 2x107 and 1x107) achei que fossem 4 doses mixed with mouse blood. Our data demonstrated that *Lu. longipalpis* is capable of sustaining infection by *L. infantum chagasi, L. amazonensis* and *L. major*, but not by *L. braziliensis*, which only developed infection when the vector ingested a high dose of parasites and even so, with low parasitic density. We also showed that infection rates of the *Lu. longipalpis* are correlated with the amount of ingested parasites. This study characterizes important aspect of the *Lu. longipalpis* vector competence.

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PRELIMINARY CHARACTERIZATION OF POTENTIAL SAND FLY VECTORS IN LEISHMANIASIS AND BARTONELLOSIS ENDEMIC AREAS IN THE PERU-ECUADOR BORDER

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Leishmaniasis and bartonellosis are known to be endemic in the Peru-Ecuador border, yet information about the sand fly vector species incriminated in transmission of both diseases is lacking. A major bartonellosis outbreak was reported in the Peruvian side of this region in 2013-2014 with 622 cases reported in Piura department and 159 cases in Cajamarca province. Concurrently, 417 and 85 leishmaniasis cases were reported in Piura and Cajamarca, respectively. The goal of this study was to characterize the sand fly fauna and potential bartonellosis and leishmaniasis vectors in sites with the highest prevalence of both diseases in this region: Lalaquiz (Piura; 1027 m.a.s.l) and Namballe (Cajamarca; 1197 m.a.s.l). Sand flies were collected in July 2015 and January 2016 using standard CDC light traps, CDC blue LED traps, CDC UV traps and Mosquito Magnet trap. A total of 464 adult *Lutzomyia* sand flies were captured; 22 from Lalaquiz (5%) and 442 from Namballe (95%). Standard CDC light trap and Mosquito Magnet trap collected the highest proportion of sand flies in Lalaquiz (45%) and Namballe (59%). The most abundant sand fly species collected from Lalaquiz were *Lu. castanea* (23%) and *Lu. shannoni* (23%); other species included *Lu. ayacuchensis*, *Lu. gomezi* and members of the Verrucarum and Micropygomyia Groups. *Lutzomyia maranonensis* (38%), *Lu. robusta* (35%), and *Lu. castanea* (27%) were recorded in Namballe. Differences in sand fly species composition between sites may be related to ecological factors. Our preliminary results suggest that different species could play a role in leishmaniasis and bartonellosis transmission at study sites. In Lalaquiz, *Lu. ayacuchensis* and *Lu. gomezi* are potential vectors of both diseases, while in Namballe, the potential vectors are *Lu. maranonensis* and *Lu. robusta*. Future studies will include assessing *Leishmania* and *Bartonella* infection rates to further characterize potential regional vectors of both diseases and contribute entomological risk information for military and civilian populations in the Peru-Ecuador border.

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NUTRITIONAL STATUS, FREQUENCY OF HUMAN BLOOD MEALS, AND *TRYPANOSOMA CRUZI* INFECTION OF *TRIATOMA DIMIDIATA* IN YUCATAN, MEXICO

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In Yucatan, the causative agent of Chagas disease, *Trypanosoma cruzi*, is transmitted by the hematophagous bug *Triatoma dimidiata*. In order to evaluate human/vector/parasite contact, and clarify the parasite cycles of transmission in the region, we are currently assessing by PCR the presence

of vertebrate and human blood meals, and the presence of the parasite in *T. dimidiata* specimens collected in sylvatic and domestic/peridomestic ecotopes in 3 rural communities of Yucatan. At this time, 222 bugs (100 males, 113 females, and nine 5th instar nymphs) have been tested for infection with *T. cruzi*. Of them, 33% were infected; infection prevalence did not differ significantly between males (33%, 33/100) and females (35%, 39/113) (χ^2=0.05, p=0.8); 22% of nymphs (2/9) were found infected. Infection rate of sylvatic bugs (45%, 34/75) was significantly higher than infection rate of (peri)domestic bugs (27%, 40/147) (χ^2=7.4, p=0.007). This may be due to different blood feeding sources between sylvatic and (peri)domestic bugs. Vertebrate blood meals were detected in 45/62 (73%) bugs tested. Of the 45 detected blood meals, 10 (22%) were human blood meals. All human blood meals were detected in (peri) domestic bugs. Thirty percent of human-fed bugs were infected with *T. cruzi*, confirming the risk of parasite transmission to human. Cloning and sequencing is now been used to identify the different vertebrate blood meal sources, and possible associations between *T. cruzi* infection and the different vertebrates. At this time, *Homo sapiens* (human), *Canis lupus familiaris* (dog), and *Zenaida macroura* (Mourning dove) have been identified. This work will help us to better understand the feeding behavior of *T. dimidiata* and to accurately describe the parasite transmission cycles occurring in Yucatan, Mexico.

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THE *LUTZOMYIA LONGIPALPIS* MICROBIOTA ASSOCIATED WITH INFECTION BY *LEISHMANIA INFANTUM CHAGASI*

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Lutzomyia longipalpis is the main vector of *Leishmania chagasi*, which is a protozoan transmitted by sandfly bite. During blood feeding on infected vertebrates, sandflies can ingest the parasites. In the midgut, the parasites interact with the native microbiota. This interaction may contribute to the metabolism of the insect providing resistance against natural enemies and parasites and also, affects the immune and reproduction. The microbiota diversity in sandflies is well known but their role on parasite infection is still not clear. Our aim was to identify the microbiota role associated with *Leishmania* infection in the sandfly *L. longipalpis*. The microbiota diversity was determined by metagenomic method using next generation sequencing (NGS) of the 4 sandfly experimental groups: sugar-fed, blood -fed, infected-blood fed and gravid. We used the multivariate method NMDS to analyze the results. It was observed that the two groups of sandflies that were fed on blood separated from the other two other groups. This observation was firstly based on the abundance of bacterial family profiles. However, the Multivariate Analysis using PCA showed the similar profile. Throughout these analysis, it was possible exclusively identify in the blood-fed sandflies the bacteria family Xanthomonadaceae. Distinctly, in the infected-blood fed sandflies, it was identify the following families: Enterobacteriaceae, Enterococcaceae, Bacteroidaceae, Coxiellaceae, Flameovirgaceae, Deferribacteraceae, Glycomycetaceae. Our results demonstrated that the bacterial community in blood-fed differs from infected blood-fed *L. longipalpis*. In vivo studies are necessary in order to show how bacteria interfere in the growth of the parasite.

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A REVERSE TRANSCRIPTASE PCR ASSAY FOR THE IDENTIFICATION OF *DIROFILARIA IMMITIS* INFECTIVE (L3) LARVAE IN MOSQUITOES

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Dirofilariasis, a mosquito-transmitted disease, is caused by parasitic nematode worms from the genus *Dirofilaria*. *Dirofilaria immitis* is principally associated with infections of domestic canines and felines but it is also capable of infecting a wide-range of mammalian species including humans. Current molecular assays for detecting *D. immitis* DNA in mosquitoes by PCR are unable to differentiate between infected mosquitoes that contain any stage of parasite DNA and infective mosquitoes that harbor third-stage larvae (L3), the larval stage capable of establishing infections in canines and other mammals. *D. immitis* has the potential to be a major public health concern due to its distribution in tropical, subtropical, and temperate regions around the world. However cases of human dirofilariasis are likely under reported due to a dearth of knowledge concerning the epidemiology and risk factors associated with this disease. The ability to identify mosquitoes that are both competent vectors of *D. immitis* and could infect humans (based upon known host feeding preferences), could play an important role in estimating transmission risks to humans. We have developed a detection assay for *D. immitis* in a model system using the mosquito, *Aedes aegypti*, based on a conventional RT-PCR assay that detects an L3-activated gene transcript. Potential L3 genes were identified using bioinformatics tools and were screened by RT-PCR using *D. immitis* stage-specific mRNA libraries as templates. Candidate genes were screened for stage-specific expression using RNA isolated from both *D. immitis* infected and uninfected mosquitoes. This L3 specific gene combined with a *D. immitis* specific control gene, expressed in all vector-stage filarial larvae, can be utilized to accurately identify mosquito species capable of transmitting this zoonotic disease.

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ARE THE POPULATIONS OF *TRIATOMA INFESTANS* FROM SANTIAGO DEL ESTERO, ARGENTINA, RESISTANT TO PYRETHROIDS

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Under the vector surveillance and control program for *Triatoma infestans* that Mundo Sano has in place since 2002 in the urban and surrounding rural areas of Añatuya (Santiago del Estero Province), monitoring of insecticide resistance was implemented in 2014. The objective was to evaluate the susceptibility of *T. infestans* samples from this area to the pyrethroid deltamethrin, active ingredient found in the most widely used formulations for triatomine control. Bioassays were performed following the World Health Organization protocol for the evaluation of insecticidal effect on triatomines. First instar nymphs obtained in the laboratory from adult insects collected in the field (F1 generation) were used. Mortality was evaluated 24 hours after topical application of the discriminant dose (2 ng/insect). The CIPEIN and La Pista (resistant to deltamethrin) strains were used as negative and positive controls, respectively. Possible resistance was considered when survival was observed in at least one insect in two of every three independent assays. Percent mortality for the different collection sites were: El Desvio 90%; Barrio Sportivo, 75%; Miel de Palo, 70%; Lote 47, 38%. These results suggest that some of the populations evaluated present early-stage resistance to deltamethrin. New assays will be conducted in order to quantify this phenomenon with greater precision.

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ANOPHELES COUSTANI, AN IGNORED SUPER VECTOR OF ARBOVIRUS AND *PLASMODIUM*

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Human malaria cases increased in Madagascar despite the use of indoor residual spraying and long lasting insecticide-treated bed nets. Vectors diversity and abundance over the year explained the persistence of malaria. Amongst 7 *Anopheles* malaria vectors including An. funestus and

An. gambiae s.s., *An. coustani* attracted the attention. During the past few years and in different seasons, *An. coustani* was regularly found positive with *Plasmodium* sp. in different ecotypes.. It was quite surprising to find this mosquito species naturally infected either by *P. falciparum* or *P. vivax*, and sometimes also with a co-infection *P. falciparum* / *P. vivax*. Moreover, during the Rift Valley Fever (RVF) outbreaks occurred in Madagascar in 2008 and 2009, *An. coustani* was also detected positive with other two species, Culex antennatus and An. squamosus/cydippis. This study aims to highlight the exact role played by *An. coustani* facing the Plasmodium and RVF virus transmission. To achieve this objective, experimental infections were developed in a high malaria prevalence area. In parallel, study on vector competence of *An. coustani* and *Cx. antennatus* to RVF virus was carried out in laboratory. We suspect that *An. coustani* played, and still playing an important role in malaria transmission whereas 14.1% of *An. coustani* and 7.23% of *Cx. antennatus* were able to transmit RVF virus in laboratory. The role of *An. coustani* in transmission of human *Plasmodium* and RVF virus was highlighted in field and in laboratory. These findings coupled with its tendency to bite on animal with an opportunistic to human confirmed its medical and veterinary importance. Otherwise, this species was found involved in transmission of *Wuchereria bancrofti* and West Nile virus in Madagascar and in transmission of Zika virus in Africa. Thus, specific attention of the biology study of this species must be carried out in order to control it and to determine its role during inter epidemic period.

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SCREENING THE MIDGUT BACTERIAL COMPOSITION OF TWO COLOMBIAN FIELD-COLLECTED MALARIA VECTORS FOR BIOCONTROL STRATEGIES

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Malaria constitutes a relevant problem of public health in Latin America with more than 300,000 cases confirmed every year in Brazil, Venezuela and Colombia. Although the number of deaths has decreased worldwide, the dramatic increase in insecticide-resistant *Anopheles* populations has led to search for alternative strategies to diminish or eliminate malaria vector populations. Recent studies have shown that some bacteria present in the mosquito microbiota have important negative effects on the sexual stages of the parasite within the mosquito midgut, as well as in vector survival. However, little is known about the microbiota of Latin American anopheline mosquitoes and its significance for parasite inhibition. Therefore, the purpose of this study is to characterize the midgut microbiota composition of two main Latin American malaria vectors, *Anopheles darlingi* and *An. nuneztovari*, collected in two malaria-endemic regions of Colombia. Metadata generated for adult mosquitoes, larvae and breeding sites by Illumina sequencing is currently under analysis. In addition, preliminary data resulting from culture-dependent methods shows interesting differences in the diversity profiles in the bacterial community of female adult mosquitoes of the two localities and species. A combined analysis of both culture-dependent methods and high throughput sequencing will help to reveal a detailed composition of the midgut microbiota of these two main vectors and elucidate potential candidates for future vector biocontrol strategies.

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DISTRIBUTION AND HABITAT CHARACTERIZATION OF *ANOPHELES* LARVAE IN FOUR COMMUNITIES IN THE PERI-IQUITOS REGION OF AMAZONIAN PERU

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Malaria control focused on drug treatment and insecticide-impregnated nets, or insecticide residual spraying, is increasingly compromised by insecticide resistance in many endemic regions. One consequence is renewed interest in the reduction of adult vector populations by targeting aquatic immature stages. Aquatic stages determine abundance, dynamics and fitness of mosquito adults, directly affecting malaria transmission. To evaluate the characterization and distribution of anopheline breeding sites, collections are being carried out in two riverine sites, Lupuna and Santa Emilia, and two highway sites, Triunfo and Nuevo Horizonte during the rainy season (Jan-Feb; April-May) and the dry season (Aug-Sept). Sampling of breeding sites is conducted using a standard dipping technique within a 1 km radius of the center of each village. Quantification includes relative abundance of larvae and Larval Index (LI) (larval density). Breeding sites will be sampled and analyzed for: concentration of nitrates and nitrites, alkalinity, pH, temperature, conductivity, salinity, turbidity, water movement, algae, density of surrounding vegetation, relative shade, and emergent light and canopy coverage. In Jan-Feb, 2016, of 32 sampled breeding sites, *Anopheles* larvae were detected in 5. Positive breeding site types were fishpond, stream margin, pond and swamp. Of 255 *Anopheles* larvae collected, An. darlingi represented 37% (96 larvae). In Lupuna 2 breeding site types were identified: stream margin (larvae=45) and swamp (larvae=65); in Santa Emilia, only pond was positive (larvae=71). In Nuevo Horizonte, larvae were found exclusively in fishponds (larvae=51); in Triunfo only pond was positive (larvae=51). Mean quantitative characteristics of the positive breeding sites were: pH = 5.5, conductivity = 29.9 ppm, salinity =4.1%, and turbidity = 20 JTU. Collected larval stages were: I = 32, II = 66, III = 92, IV = 59, and 6 pupae. Descriptive characteristics were partial shade, moderate water movement, and moderate submerged and emergent aquatic vegetation. These characteristics can determine presence of *Anopheles* larvae in the habitats.

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CHARACTERIZATION OF LARVAL HABITATS OF *AEDES AEGYPTI* IN KENYA

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Aedes aegypti, the principal vector for dengue and other emerging arboviruses, breeds preferentially in various man-made and natural container habitats. In the absence of vaccine, vector control is the primary means to reduce the incidence of dengue. Effective vector control depends on a good understanding of larval and adult vector ecology of which little is known in Kenya. Twenty sentinel houses in each of four study sites (in western and coastal Kenya) were assessed for immature mosquito incidence once a month for a period of 20 months (May 2014

to December 2015). All water-holding containers in and around the households were inspected monthly for immature *Ae. aegypti* mosquitoes. A total of 19,249 containers were inspected from Chulaimbo (6929) and Kisumu (6927) in the west, and from Msambweni (2689), and Ukunda (2704) on the coast. Of these, only 5.8%, 5.3%, 5%, and 6.6%, respectively, were positive for *Ae. aegypti* immatures. In all four sites, significantly more positive containers were located outdoors than indoors (χ^2= 712.4, DF=1, P<0.001). A total of 12,547 *Ae. aegypti* immatures were collected from these containers, which comprised 13 container types. More than 40% were from buckets, tires, and water-tanks, which produced 49% (1,245/2,530) of the pupae in the western and coastal study sites combined. Tanks, buckets, drums, and flowerpots were the key indoor containers, producing > 80% (92/108) of the pupae. Key outdoor containers in the coast were tires, tanks, buckets, and basins which accounted for 57% (1,316/1,965) of pupae, while pots and tires were the only key containers in the western region producing 71% (329/457) of pupae. Coast region produced significantly more *Ae. aegypti* immatures than the western region (Kruskal-Wallis, χ^2 = 179.8, DF=1, P< 0.0001). These results indicate that *Ae. aegypti* breeding habitats are abundant outdoors and are diverse both in the coast and western regions of Kenya. However, only a few containers are responsible for majority of the production. Targeting source reduction efforts towards these productive containers may be a cost-effective way to reduce dengue transmission in these regions.

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XENOMONITORING OF *WUCHERERIA BANCROFTI* INFECTION BEFORE AND AFTER MASS DRUG ADMINISTRATION IN DREKIKIER, PAPUA NEW GUINEA

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Xenomonitoring using entomological techniques is useful for measuring filarial infection in human populations indirectly through mosquito collections. Since the introduction of mass drug administration (MDA) and vector control programs, microfilaria prevalence in human populations is decreasing to thresholds where transmission may no longer be sustainable. The human landing catch is the standard used in calculating transmission indices where large numbers of mosquitoes can be collected to estimate disease prevalence and monitor transmission of *Wuchereria bancrofti* filarial worms in post-MDA programs. In this study, we monitor bancroftian infection in mosquitoes pre- and post-MDA using 2014 and 2015 collections from East Sepik, Papua New Guinea. Monitoring was conducted in three representative villages of high and moderate transmission zones within the Drekikier District. In the two representative villages of high transmission, 293 samples were collected in 2014, and 448 samples were collected in 2015. In the representative village of moderate transmission, 43 samples were collected in 2014, and 61 samples were collected in 2015. The samples were collected using human landing catch conducted from 6pm to 6am each night for six nights before and after one round of MDA. Results show 1.5 times more infection in the moderate transmission zone than in the high transmission zone pre-MDA but 0.6 times lower in post-MDA. This could be explained by a roughly 7-fold higher man biting rate in the high transmission zone compared to the moderate transmission zone (24.4±3.6 vs. 3.6±1.5 in 2014 and 74.7±14.8 vs. 10.2±2.9 in 2015). Sample DNA was extracted, run using conventional PCR and viewed through gel electrophoresis to find the mosquito infection rates. The mosquito infection rates of *W. bancrofti* decreased in both high (10.8%±0.21% to 2.6%±0.07%, p<0.0001) and moderate (16.3%±1.7% to 1.6%±0.4%, p=0.0082) transmission zones. Xenomonitoring of mosquito collections done before and after one round of MDA correspond with decreases in human infection by light microscopy (28.8% to 9.0%, p< 0.0001) in one representative village after one round of MDA.

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ASSESSING THE SPATIAL HETEROGENEITY OF MALARIA VECTORS IN THE CONTEXT OF INCREASING VECTOR CONTROL INTERVENTIONS

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Environmental variation across a landscape naturally contributes to spatial heterogeneity in malaria risk, yet the coverage of malaria interventions is increasing dramatically across many endemic regions. Novel geostatistical methods to investigate the spatio-temporal heterogeneity of malaria are increasingly available in open-source software. Investigating the spatio-temporal heterogeneity of malaria vectors is critical for understanding how transmission patterns change in the context of increasing intervention coverage. In this study we investigated the spatio-temporal heterogeneity of host-seeking adult mosquitoes in a region of southern Malawi where malaria interventions are being intensively scaled-up. We used geostatistical methods to efficiently sample houses for mosquitoes at a sub-district level. Host-seeking mosquitoes were collected using Suna traps on a continuous, rolling basis for one year. We used model-based geostatistics to account for spatial correlation in assessing the determinants of mosquito distribution, and to map spatial variation in the abundance of mosquitoes. Preliminary findings suggest clear spatial patterns in mosquito abundance. The results provide a basis for determining the spatio-temporal heterogeneity of malaria in the context of a community-based, vector-control intervention trial.

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BITING BEHAVIOR OF *ANOPHELES ALBIMANUS* IN ARTIBONITE, GRAND'ANSE AND NORD-EST, HAITI

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Hispaniola is the only island in the Caribbean where malaria transmission still occurs, with Haiti having a higher number of malaria cases than the Dominican Republic (DR). While both Haiti and DR are committed to eliminating malaria by 2020, no studies on mosquito behavior have been conducted on the island since 1988. We conducted studies to determine human exposure to *Anopheles albimanus* bites indoors and outdoors during the night in three different regions in Haiti: Artibonite, Grand'Anse and Nord-Est. Mosquitoes were collected by all-night human landing catches to determine man-biting rates and the timing of bites. *A. albimanus* showed preference to bite more often outdoors than indoors in all three sites: the outdoor-to-indoor biting ratio (O/I) was 1.10 (95% CI: 1.03 – 1.18) in Artibonite, 1.72 (95% CI: 1.59 – 1.87) in Grand'Anse and 1.88 (95% CI: 1.13 – 3.92) in Nord-Est. However, when weighting the biting rate against the time people reported being in bed, there was a higher exposure of mosquito bites to people indoors than outdoors in Artibonite (weighted O/I = 0.11; 95% CI: 0.11 – 0.13) and Grand'Anse (weighted O/I = 0.51; 95% CI: 0.41 – 0.61) but not Nord-Est (weighted O/I = 1.36; 95% CI: 0.57 – 3.40). These results suggest that indoor mosquito control methods such as indoor residual spraying or insecticide treated bednets may be effective in preventing malaria transmission in some areas

of Haiti where most people are indoors during the early evening. However, outdoor vector approaches may still be required to support malaria elimination efforts in Haiti.

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CHARACTERIZING TEMPERATURE IN LOCAL MALARIA TRANSMISSION ENVIRONMENTS

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The key mosquito and parasite life history traits that combine to determine malaria transmission intensity are all affected by temperature. To understand transmission ecology, therefore, it is important to determine the range of microclimatic temperatures experienced by malaria vectors in the field. This study was conducted in 6 rural villages of Sundargarh District in Odisha, India. Within these villages, data loggers were used to record microclimates in multiple locations and structures. These microclimate data were then used to drive a malaria parasite development model to compare predictions of parasite extrinsic incubation period (EIP) under local conditions (we use EIP here as an illustrative trait to explore the biological consequences of variation in local microclimate). Mean temperatures and temperature variation differed between resting sites within the transmission environments. Mean temperatures were around 2°C higher inside asbestos roofed houses than in outdoor vegetation, with tiled houses and cattle sheds intermediate to these extremes. Diurnal temperature variation was much greater outdoors compared with variation measured within domestic dwellings. Exploring the effects of temperatures on malaria parasite development rate revealed negligible differences between mircohabitats during the warmest times of the year (i.e. EIPs of around 10 days irrespective of environment). However, under cooler times the predicted EIPs varied substantially, being 10 or 20 days longer in vegetation, tiled houses or cattle sheds, than asbestos houses. Moreover, for large parts of the year, the EIPs predicted for the specific microhabitats differed to those predicted using weather station data. The current study reveals that microclimates can vary substantially between habitats within local transmission environments. Small differences in microclimate can potentially lead to large differences in life history traits. Measuring the range of conditions available to mosquitoes within local transmission settings should provide a more robust characterization of transmission ecology than remote weather station data.

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MEASURING SPECIES - AND REGION-SPECIFIC MARKERS OF MOSQUITO BITES BY SMALL PEPTIDE ARRAYS

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Knowledge about human exposure to mosquitoes can help target interventions to those at greatest risk of malaria and other vector-borne diseases. Mosquito trapping estimates local vector populations, but implementation of large scale collection programs is difficult, and mosquito presence alone does not indicate who is being bitten. Measuring antibody responses to mosquito salivary antigens is a potential strategy to estimate exposure to specific vectors, locations and risk groups. Two *Anopheles* salivary antigens, SG6 and cE5, have been shown to elicit a humoral immune response in humans that can be measured by ELISA to indicate recent exposure to mosquito bites. However, due to the conservation of these genes, ELISAs have limited ability to discriminate between exposures to different vector species. We developed a small linear peptide array to measure antibody responses to individual epitopes along the full length of six mosquito salivary proteins each from six *Anopheles* species. The arrays were probed with sera from West African children and adults, Southeast Asian adults with acute falciparum malaria, and North American adults, allowing us to observe differences in the antibody binding profiles of people to antigens from geographically separated mosquito species. Southeast Asian adults reacted most strongly to peptides from Southeast Asian mosquitoes and West African sera reacted most to peptides from African mosquitoes, while North American adults had the lowest seroreactivity to all six *Anopheles* species. From these data, we identified a set of peptides that can differentiate between the sera of people bitten by mosquitoes from different regions. The set of mosquito proteins on the array can expand to include more salivary proteins and mosquito species, allowing us to build a panel of peptides to measure human exposure to diverse vectors of a variety of mosquito-borne diseases. These peptides could potentially be used to develop a point-of-care test to estimate human exposure to mosquitoes allowing for better targeting of vector-borne disease interventions.

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ENVIRONMENTAL FACTORS INFLUENCING *ANOPHELES DARLINGI* POPULATION DYNAMICS AND MALARIA TRANSMISSION IN ZUNGAROCOCHA, LORETO, PERU

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A sustained increase in the number of malaria cases has been observed in Peru since 2012 with the majority of cases reported in Loreto, northern Amazon, where *Anopheles darlingi* is the dominant malaria vector. Examining *An. darlingi* population densities, age structure, and natural *Plasmodium* infection rates in this malaria endemic region is essential for malaria transmission risk assessment. Moreover, the impact of environmental factors on *An. darlingi* abundance and malaria transmission could further contribute to understand malaria transmission patterns. This study aimed to estimate *An. darlingi* seasonal abundance, age structure, and *Plasmodium* infection rates; and correlate with local environmental parameters and malaria cases reported by the Ministry of Health in Zungarococha, a malaria-endemic community in Loreto. *An. darlingi* densities were estimated by protected Human Landing Catch performed outside local homes from 1800h to 0600h for a 3-day period each month from June 2014-March 2016. A subset of *An. darlingi* females (30%) was dissected to determine parity status and remaining specimens were stored for *Plasmodium* spp. detection. Local temperature, humidity and rainfall were simultaneously recorded. A total 15,904 *An. darlingi* females were captured; 5,123 were dissected. *An. darlingi* densities ranged from 0.5 to 164 mosquitoes landing per person per hour, with the highest densities (>100) recorded in June 2014 (111), January-March 2015 (123-127), July 2015 (117) and January 2016 (164). Parous was the predominant ovarian stage in mosquitoes dissected (>60%) during density peaks. The number of malaria cases reported monthly ranged from 62-285, with highest numbers (>150 cases) in June-July 2014 (175-214), July-September 2015 (152-157), and Feb-March 2016 (157-285), a few weeks after mosquito population density peaks and heavy rainfall. *An. darlingi* densities shows a positive association with rainfall events. Results are discussed in terms of the impact of environmental factors on vector population and malaria transmission dynamics, and will help guiding vector control strategies in the region.

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ASSOCIATION OF AUTOCIDAL GRAVID OVITRAPS WITH REDUCED RATES OF CHIKUNGUNYA VIRUS INFECTION IN PUERTO RICO

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There are currently no effective and sustainable interventions to prevent infections with viruses transmitted by *Aedes* mosquitoes. Since 2012, four communities in Puerto Rico have been participating in a field trial of a recently developed autocidal gravid ovitrap (AGO). After 3 AGO traps were placed in >85% of homes in two intervention communities, adult *Ae. aegypti* mosquito populations were reduced by ~80% compared to two non-intervention communities. The introduction of chikungunya virus (CHIKV) to Puerto Rico in May 2014 provided an opportunity to determine if AGO traps were associated with CHIKV infection rates in humans and mosquitos in these communities. To estimate the seroprevalance of CHIKV infection in intervention and non-intervention communities, 377 houses were randomly selected. Participating household members provided a blood specimen and completed a questionnaire on demographics, recent illnesses, and mosquito avoidance practices. Serum specimens were tested by IgG ELISA to detect historic CHIKV infection. During November 2015 and February 2016, a total of 233 (62%) households from the four communities agreed to participate. Mean age of participants (53 years) was greater than that of all eligible residents (49 years). Mean age of participants from intervention communities was not significantly different from those from non-intervention communities. Among 152 and 175 participants from non-intervention and intervention communities, historic CHIKV infection was detected in 69 (45%) and 40 (23%) participants, respectively. The observed two-fold difference in the prevalence of CHIKV infection in intervention compared to non-intervention communities may be associated with the lower measured mosquito densities in communities where AGO traps are present. Additional analyses are being performed to adjust anti-CHIKV antibody prevalence with respect to sampling design, community differences, and participation rates. These findings may implicate AGO traps as an effective and sustainable community intervention to prevent infections transmitted by *Ae. aegypti* mosquitos.

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BACTERIAL COMPOSITION OF LARVAL BREEDING SITES OF AFRICAN *AEDES AEGYPTI* AND ITS EFFECT ON VECTORIAL CAPACITY

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Like other animals, insects establish symbiotic associations with microbial communities that shape their individual phenotype and fitness. In particular, the native gut bacteria of insect vectors can modulate their immunity and susceptibility to human pathogens. Compared to vertebrates, insects have more labile gut bacterial associations that are under strong influence of the environment. Thus, habitat-related differences in bacterial communities could mediate an environmental influence on vector-borne pathogen transmission. We investigated whether differences in the bacterial communities of larval breeding sites may drive variation in vectorial capacity of the mosquito *Aedes aegypti*, a major vector of dengue, Zika, and chikungunya viruses. In Sub-Saharan Africa, *Ae. aegypti* larvae develop both in domestic habitats such as human-associated containers and in sylvatic habitats such as rock pools or tree holes. Comparison of natural sylvatic and domestic breeding sites in Gabon by metataxogenomics revealed contrasted bacterial communities in the water and, to a lesser extent, in the midgut of adult *Ae. aegypti* emerging from these breeding sites. To test whether exposure to different bacteria during larval development may differentially affect adult vectorial capacity, we created gnotobiotic larvae using a selection of four bacterial isolates from the natural breeding sites in Gabon. Mono-association with *Enterobacter*, *Salmonella*, *Arthrobacter*, or *Rhizobium* bacterial isolates during larval development resulted in significant differences in pupation rate. In addition, larvae exposed to the *Arthrobacter* isolate had larger bacterial loads in adult midguts pre and post blood meal, showed decreased antibacterial activity in adult hemolymph, and were less susceptible to dengue virus infection. No differences in adult lifespan were detected between the different gnotobiotic treatments. Together, our results provide the proof of principle that habitat-related differences in larval exposure to bacteria can drive variation in adult mosquito immunity and vectorial capacity.

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THE IMPORTANCE OF HUMAN POPULATION CHARACTERISTICS IN MODELING MOSQUITO VECTORS: A COMPARATIVE ANALYSIS OF MODEL COMPONENTS

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The current Zika virus epidemic in the Western hemisphere is representative of the confluence of global climate change and infectious disease expansion, and vector modeling represents a pertinent and timely method to analyze the environment associated with Zika-carrying mosquitoes. Among many mosquito species distribution models, there are varying opinions on which variables are most predictive and, consequently, should be included in modeling efforts. While climate variables (e.g., mean temperature, mean precipitation) are routinely included, some argue that human population dynamics, in the form of population density and socioeconomic status, should also be included. This project aimed to test the importance of including human population characteristics by modelling the Zika virus vector *Aedes aegypti* in the Southeastern United States with climate variables, population density, and poverty characteristics. A. aegypti occurrences, global climate data, and population characteristics were obtained from publicly available sources and sampled at a resolution of 2.5 arc-minutes. Data pre and post-processing was completed in ArcMap 10.3 and models were created in Maxent v.3.3.3k. Four models were developed for this project: a climate-only model, a climate and population density model, a climate and poverty model, and a combined model with climate, population density, and poverty. Models were evaluated by comparing test and training area under the curve metrics, omission and commission errors, and variable jackknifing results. The climate-only model performed poorly compared to models with human population characteristics. The combined model was the best fit, though the model with climate and population density had a lower commission rate (21.0% and 20.6%, respectively). Jackknife results for the full model showed that population density was the most significant contributor to the model. This research indicates that more consideration should be given to human population characteristics when modelling mosquito habitats.

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URBAN MICROCLIMATE AND DENGUE VECTOR COMPETENCE OF THE INVASIVE ASIAN TIGER MOSQUITO, *AEDES ALBOPICTUS*

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Vector-borne diseases have increased in urban environments in recent decades. Incidence of vector-borne disease is often unevenly distributed across urban landscapes, which are themselves comprised of a diversity of landscape features that can modify thermal microclimates. Because mosquitoes are small ectotherms, their growth, survival, and reproduction are all sensitive to fine-scale variation in microclimate. Additionally, microclimate during the larval stage can affect life history traits of adults, a phenomenon known as carry-over effects. The majority of studies assume temperature to be the most important factor driving carry-over effects, enabling research to be done in the lab. However, laboratory research is unable to fully incorporate realistic field microclimate conditions experienced by mosquito vectors. To further explore the effect of larval microclimate on Ae. albopictus vector competence, we conducted a semi-field experiment examining vector competence across urban, suburban, and rural sites in Athens, GA. *Aedes albopictus* larvae were reared in the field across the three treatments, and offered a DENV-2 infectious bloodmeal post-eclosure, to measure the effect of microclimate on dengue infection, dissemination, and transmission. Information on vector competence was then incorporated with mosquito life history, potential larval habitat density, and adult abundance data collected from the same sites, to better characterize risk of dengue through vectorial capacity across the urban gradient.

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A TRADE-OFF BETWEEN DRY SEASON SURVIVAL LONGEVITY AND HIGH WET SEASON NET REPRODUCTION EXPLAINS THE PERSISTENCE OF *ANOPHELES* MOSQUITOES

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Plasmodium falciparum malaria remains a leading cause of death in tropical regions of the world. Despite efforts to drive transmission down, rebound epidemics associated with the persistence of malaria vectors have remained a major impediment to local elimination. One area that remains poorly understood is how *Anopheles* populations survive long dry seasons to re-emerge following the onset of the rains. We developed mathematical models to explore the impact of different mosquito survival strategies on the dynamics of the vector population. We show that mosquitoes have different lifestyles between the wet season and the dry season. Their ability to persist is attributed by their propensity to exploit the wet season (fast and high reproductive output), but then mitigate the effects of the dry season (longevity and aestivation). We demonstrate that aestivation is a population rescue strategy that makes ecological vector population extinction difficult, while wet season high reproductive output buffers the population against dry season potential extinction. We show that both longevity/aestivation and high wet reproduction allow persistence of the mosquitoes, and can reproduce patterns observed in field data from the Sahel region. Our results demonstrate the importance of practical ecological methods to control vectors in the dry and wet seasons if malaria transmission is to be interrupted.

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DENGUE FEVER AND *AEDES AEGYPTI* RISK IN THE GALAPAGOS ISLANDS, ECUADOR

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Dengue fever, chikungunya and zika virus, transmitted by the *Aedes aegypti* mosquito, are emerging infectious diseases in the Galapagos Islands of Ecuador. In 2014 we conducted a pilot study on two islands (Puerto Ayora (PA) on San Cristobal and Puerto Baquerizo (PB) on Santa Cruz) to assess *Ae. aegypti* abundance, key larval habitats and household risk factors for dengue infection. We surveyed 100 households (50 per island) in high-risk areas. Adult *Ae. aegypti* were collected inside and outside the home using prokopack aspirators, larval indices were determined through container surveys, and heads of households were interviewed to determine demographics, housing conditions, and knowledge, attitudes and practices regarding dengue. Multimodel selection methods were used to identify best-fit logistic regression models to explain the presence of *Ae. aegypti* and self-reported prior dengue infections. We found that 24.3% of PB and 14% of PA participants self-reported a prior dengue infection. The best-fit model to explain prior infection indicated higher risk for people who frequently traveled between the islands, households that experienced interruptions in the piped water supply, and heads of households with salary above the minimum wage. Adult *Ae. aegypti* were collected from 14% of PB and 4% of PA houses; other adult mosquitoes collected included *Culex quinquefasciatus* and *Ae. taeniorhynchus*. Significantly more PB homes than PA homes had containers with *Ae. aegypti* juveniles ($p = 0.012$; PB House Index = 20, Breteau Index = 26; PA House Index = 6, Breteau Index = 6). The characteristics of the predominant *Ae. aegypti* larval habitat were 55-gallon water storage drums, located outdoors, uncovered, shaded, and filled with tap-water. The best-fit model to explain the presence of *Ae. aegypti* indicated higher risk in households that used tanks for water storage and in households that perceived that dengue prevention was difficult. These findings provide the region's public health sector with key information for conducting dengue and zika control campaigns, and highlight the importance of local socio-ecological studies to understand dengue risk.

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DYNAMICS OF INGESTED *ENTEROBACTER* SP. IN THE GUT OF MOSQUITOES THROUGHOUT LIFE CYCLE

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There is a symbiotic relationship between the mosquito and its gut microbial residents. Taxa in genus Enterobaacter are commonly present in the gut of various mosquitoes. But few data were available regarding the dynamics of bacteria from being ingested to being egested. In this study the strain Ag1 of Enterobacter sp. was tagged with green fluorescent protein (GFP), and its dynamics were tracked in the gut of both *Anopheles gambiae* and An. stephensi. GFP tagged bacteria were provided to mosquitoes in the sugar meals for two days. Introduced bacteria were visualized by imaging individual gut under a fluorescent microscope. The bacterial intensity was estimated by average number of fluorescent colonies per gut. In the sugar fed guts, the prevalence of bacteria

decreased over time from 100% to 37% in *An. gambiae* on day 6 post ingestion and 30% in An. stephensi on day 6 post ingestion. At this time point, mosquitoes were given a blood meal, which brought prevalence back to ~70%. The decline of bacteria in the gut is correlated with the presence of GFP-bacteria in the feces, indicating that the bacteria were discharged through defecation. Enterobacter was favored in the blood fed guts, and proliferated well for two days, then starting being egested with digested wastes in feces. The data indicate that the bacteria are able to reside in the gut but the length of stay varies individually. Moreover, when gravid mosquitoes laid eggs in water, Enterobacter cells in feces entered into aquatic habitat. GFP bacteria were detected in the culture of larval gut homogenates of larvae and emerged adults. Evidently, Enterobacter transstadially passed through metamorphosis from larvae to adults. Mosquito associated bacteria can cycle from generation to generation through fecal-oral route.

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DRIED BLOODSPOTS FROM PAIRED HUMAN AND ENGORGED MOSQUITOES TO DEMONSTRATE THE FEASIBILITY OF XENOSURVEILLANCE IN WEST AFRICA

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Xenosurveillance is a novel technique that utilizes mosquito bloodmeals to noninvasively survey human populations for infectious diseases. The technique takes advantage of the host-seeking and blood-feeding behavior of *Anopheles gambiae* mosquitoes. Two villages in Northern Liberia were enrolled in to our study, and human dried bloodspots were collected during the initial enrollment period. The villages were subsequently sampled for indoor resting engorged mosquitoes on a rotating schedule for the following two weeks. Bloodfed mosquitoes collected from inside homes had their midgut contents expelled onto FTA cards for further testing. RNA from both human and mosquito samples were eluted, extracted, pooled, and subjected to Next Generation Sequencing. Bioinformatic analysis revealed the presence of GB virus C in both human and mosquito samples collected in the same home. A total of 15 and 28 reads aligned to West African strain of GB virus C, respectively. This resulted in a combined coverage of 40% of the genome. These matched results indicate that our technique can reliably detect genetic signatures of human viruses in mosquito bloodmeals. This study demonstrates the feasibility of Xenosurveillance in a field setting.

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ADVANCING ARBOVIRUS SURVEILLANCE BY ASSESSING THE EFFICIENCY OF VECTOR AND DISEASE MONITORING PROGRAMS

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Mosquito surveillance is a necessary component of public health for assessing human health risks for a plethora of transmittable diseases. Vector presence, abundance, and infection rates are needed to effectively manage efforts in mosquito control operations. Over the past few years, mosquito surveillance and control have become increasingly important as endemic diseases resurface, as happened West Nile virus (WNV) in 2012 in the United States, and new diseases emerge, as is currently occurring with Zika virus. However, mosquito collection and testing to monitor these diseases can become financially burdensome, especially in developing countries and sparsely population regions. Using WNV surveillance data from South Dakota, our study looks to determine whether the current level of mosquito and arbovirus surveillance can be reduced while maintaining a similar capacity to predict risk to humans. Mosquitoes were captured and tested for WNV in 27 counties for varying numbers of years from 2003 to 2015. The minimum infection rate was calculated for every county in every reported week, and the maximum MIR (MMIR) for every county-year combination was estimated in those county-years. Analysis of variance was used to determine if any significant differences could be detected among the MMIR for county-year combinations. Our model suggests that there are some persistent differences in MMIR between counties ($p = 0.01245$). A Tukey multiple comparison test showed only two counties to have a significant difference in their mean MMIR. However, MMIR correlated well with human cases, and was an effective predictor of total yearly risk. In a set of regressions, we considered whether any county could serve as a proxy for other counties or the state as a whole. We found that no individual county's MMIR was more informative than statewide MMIR. The results suggest that maintaining broader spatial coverage across the state is likely preferable to oversampling individual counties.

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COMPARATIVE SUSCEPTIBILITY BETWEEN FIVE *AEDES AEGYPTI* POPULATIONS TO FOUR DENGUE VIRUS SEROTYPES

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Dengue is the most important arboviral-infection affecting humans and in the America, its main vector is *Aedes aegypti*. There are multiple factors that are key to the successful spread and maintenance of the dengue virus cycle in nature: vertebrate host biology, circulating viral strain, vector susceptibility towards the virus. The last one, vector competence, may vary according their geographic distribution, even within the same city. Therefore our aim is assessing the existence of vector competence differences between five *Ae. aegypti* populations collected throughout the city of Manaus considering four dengue virus serotypes. *Ae. aegypti* eggs from five locations within the city of Manaus, Amazonas, Brazil were collected and reared under laboratory conditions (temperature 26°C and a relative humidity of 80%). Individuals from each mosquito population were then challenged simultaneously to the tested serotypes by membrane feeding assay. Fourteen days post infection, the mosquitoes were dissected separating the head from the body. Viral RNA extraction was performed with the QIAamp Viral RNA Mini Kit, whereas the detection and quantification of viral RNA were performed with the Power SYBR Green Kit Step-1. We then calculated the infection rate (IR) and dissemination rates (DIR), as well as the vector competence(VC) for each population. To date, our analyses showed significant differences regarding DENV-1 IR than have variation 25 to 100% and DIR range of 50 to 95%, as well in VC with a range of 12,5 to 95% between the five *Ae. aegypti* populations. Despite the high rates of infection, there was no significant difference with DENV-2 in all populations with IR varying from 90-100%, DIR 100% and VC varying from 90 to 100%. The results also showed DENV-4 with the similarity among populations, with IR ranging from 85 to 100%, DIR varying from 80 to 100% and VC with a range of 70 to 94.7%. This study we will be able us to further understand the vector- virus interaction and the dynamics of dengue transmission in a local urban context.

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HUMAN EHRLICHIOSIS AND ANAPLASMOSIS IN NORTHEASTERN THAILAND

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Human Monocytic Ehrlichiosis (HME) and human granulocytic anaplasmosis (HGA) are emerging, tick-borne rickettsial diseases. In Thailand, the first HME cases were first reported in 1997.A prospective etiologic study of patients with acute undifferentiated fever (AUF) was conducted in Maharat Nakhon Ratchasima hospital, northeastern Thailand. Serological tests for *Ehrlichia chaffeensis* and *Anaplasma phagocytophilium* IgM and IgG antibodies using commercial immunofluorescence antibody assay were performed in 70 AUF patients who tested negative for scrub typhus, murine typhus, spotted fever group, Q fever, leptospirosis, dengue, malaria, and bloodstream infection. The result showed seroprevalence (IgG) of *E. chaffeensis* and *A. phagocytophilium* in 37.1% (26/70) and 21.4% (15/70) patients, respectively. Eleven (15.7%) patients were IgG positive for both *E. chaffeensis* and *A. phagocytophilium* antigens. Additionally, 5 (7.1%) cases had positive *E. chaffeensis* IgM antibody titer at 1:64. Three confirmed HME cases were diagnosed based on clinical compatible illness and single serum of *E. chaffeensis* IgG titer ≥ 1:256. Most of them had common clinical (fever, myalgia, headache) and laboratory findings (elevated liver enzyme, thrombocytopenia). Complications developed in two patients: septic shock and acute renal failure. Two patients, one of which had acute renal failure, received ceftriaxone and doxycycline and both of them fully recovered. Another patient with septic shock received intravenous ceftriaxone and chloramphenicol and subsequently died. In conclusions, HME and HGA should be included in the differential diagnosis of Thai AUF patients and prompt doxycycline treatment is likely to improve outcome.

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DISTRIBUTION AND MOLECULAR CHARACTERISTICS OF *RICKETTSIAE* FOUND IN TICKS ACROSS CENTRAL MONGOLIA

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Little is known regarding tickborne diseases in Mongolia. A total of 1,497 adult unfed ticks; 261 *Ixodes persulcatus*, 795 *Dermacentor nuttalli*, and 441 *Hyalomma asiaticum,* were collected from the environment and off livestock returning from pasture, across a vertical stretch of Central Mongolia spanning from China to Russia (Selenge, Tov, & Dornogovi aimags). Ticks were then separated into genus specific pools (n=299), by sample location, containing ~5 ticks each. After extraction of DNA and RNA, nested polymerase chain reaction (PCR), reverse transcription-PCR (RT-PCR), and quantitative real-time RT-PCR (qRT-PCR) were conducted to detect rickettsia bacteria and tickborne encephalitis virus (TBEV). Assays yielded pool detection rates of 92.5% (49/53) and 1.9% (1/53) of *I. persulcatus* pools testing positive for *Candidatus Rickettsia tarasevichiae* and TBEV respectively, while *Rickettsia raoultii* was found in 72.8% (115/158) of *D. nuttalli* pools. Both *R. tarasevichiae* and *R. raoultii* are recognized as emerging tickborne diseases, with this being one of the first reports of *R. tarasevichiae* in Mongolia. Given that *R. tarasevichiae* shares the same vector (*I. persulcatus*) as TBEV, and may present with severe atypical spotted fever group (SFG) rickettsia-like symptoms, Mongolian physicians treating suspected cases of TBEV should include *R. tarasevichiae* infection in their differential diagnosis and consider prescribing antimicrobial therapy.

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SEROPREVALENCE OF *RICKETTSIA* AND *ANAPLASMA* EXPOSURE IN HUMANS AND LIVESTOCK ACROSS A CENTRAL STRETCH OF MONGOLIA

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Tickborne diseases (TBD) are suspected to be a major cause of illness in Mongolia, although the extent of which remains unknown. Therefore, to better understand the burden of TBD in Mongolia, serosurveillance focusing on anaplasma and rickettsial infections was carried out through an international collaboration between US and Mongolian researchers from September 2014 to October 2015. Samples were collected from 388 nomadic herders, 867 goats, 871 sheep, 367 cattle, and 216 horses, across three provinces (Selenge, Tov, Dornogovi). Serum samples diluted 1:50 in sterile 1X PBS were placed on IFA slides coated with *R. rickettsii* and *A. phagocytophilum*, and examined using a fluorescent microscope. The overall seroprevalence of anaplasma and rickettsia in human samples was 136/365 (37.3%) and 73/374 (19.5%), respectively. Anaplasma and rickettsia seroprevalence rates in livestock were 1,120/2,370 (47.3%) and 478/2342 (20.4%). Such high rates of exposure might possibly be attributed to the increased risk of tick bites associated with a nomadic lifestyle, where both herder and livestock spend most of the day traveling from pasture to pasture. Significant differences in detection rates by sample species and region were observed for both pathogens of interest, indicating a higher prevalence of exposure of TBD in the central and northern provinces of Tov and Selenge, compared to that of the Gobi region of Dornogovi. These findings lay a framework for much needed future tickborne disease research in Mongolia, while providing valuable information to veterinarians, clinicians, and policy makers currently involved in ongoing TBD control efforts.

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RAPID DIAGNOSIS OF *ANAPLASMA PHAGOCYTOPHILUM* WITH P44 DIPSTICK ASSAY

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Anaplasma phagocytophilum is tick-borne bacterial zoonosis, infects into and destroys host blood cells. Human patients show influenza-like symptoms with fever, headache, myalgia, and malaise. Human granulocytic anaplasmosis (HGA) is 3rd most common tick-borne infections in US behind Lyme disease and Rocky Mountain spotted fever. Commercial IFA kit has been used for diagnosis of canine or horse Anaplasmosis, but other animal reservoirs, such as cattle, deer, goats, have not been developed until now. Dipstick for detection of *A. phagocytophilum* antibody will be useful for early control of *A. phagocytophilum* because of on-site applicability. Therefore, dipstick kit for reservoir animals is urgently needed for early diagnosis of animal anaplasmosis. Recombinant surface protein p44 of *A. phagocytophilum* was produced by *E. coli* expression system and the immunological specificity was confirmed by Western blotting with positive control serum. Three types of dipstick kit for *A. phagocytophilum*

antibody detection were designed and compared with their reactivity, which were sandwich, indirect I using anti-bovine IgG, and indirect II using protein A. And then the reactivity of test line was also read by gold reader. Recombinant p44 protein was expressed from *E. coli*, and used as dipstick antigen. The protein size was 44 kDa and the specificity was confirmed by Western blotting. Indirect I and II could differentiate positive and negative serum, and the discriminatory power of indirect II was superior to those of other types. Dipstick kit for *A. phagocytophilum* will be useful for early detection of *A. phagocytophilum* infection at on-site farm and helpful for early control of zoonotic anaplasmosis at animal level.

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RAPID DIAGNOSIS OF *EHRLICHIA CHAFFEENSIS* WITH P120 DIPSTICK ASSAY

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Ehrlichia chaffeensis is tick-borne bacterial zoonosis, infects into and destroys host blood cells. Human patients show influenza-like symptoms with fever, headache, myalgia, and malaise. Human monocytic ehrlichiosis (HME) is 4th most common tick-borne infections in US behind Lyme disease, Rocky Mountain spotted fever, and HME. Commercial IFA kit has been used for diagnosis of human or canine Ehrlichiosis, but those for other animal reservoirs, such as deer, goats, and cattle, has not been developed until now. Antibody detection dipstick kit to *E. chaffeensis* infection will be useful for early control of *E. chaffeensis* by early detection at on-site farm. Therefore, dipstick kit for reservoir animas is urgently needed for early diagnosis of animal ehrlichiosis. Recombinant surface protein p120 of *E. chaffeensis* was produced by *E. coli* expression system and the immunological specificity was confirmed by Western blotting with positive control serum. Concentration of gold particle and dilution factor of serum were determined by Dot assay with recombinant p120 protein and positive control serum. Recombinant p120 protein was expressed from *E. coli* and used for dipstick antigen. The protein size was 43 kDa and the specificity was confirmed by Western blotting. Optimal concentration of gold particle in Dot assay was OD7 and that of serum dilution 2X dilution. Deer positive serum showed clear band on test line, and there was no reaction band to deer negative serum. Dipstick kit for *E. chaffeensis* will be useful for early detection of *E. chaffeensis* infection in on-site farm and helpful for early control of zoonotic ehrlichiosis at animal level.

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UTILIZE ALL FOR HEALTH. AN OBSERVATIONAL STUDY

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Human is the only attribute to blush good innermost emotions healthy norm of daily living activities. Human mutual cooperation, share events, happiness and benefiting the human as a whole less tendencies toward disease, deaths. Happiness more choices, more demand and increasing supply goods decreasing diseases, deaths, disparities among human. Health contribution is susceptible to sustainable variable environment. The total gross world product (GWP) is approximately US$107.5 trillion reflect purchasing power parity the per capita GWP was approximately US$16,100 in 2014. An in-depth review was performed to emerging idea on the role of the global health investors in health service utility. The articles and other documentation were then summarized to assess what is known about the investor's sector's role in global health as well as gaps in the literature. In conclusion, society health contribution system reduces the health care cost serve the wellbeing of humanity the final key to health for all. The health utility is a total multidisciplinary and multi-sectoral approach is emerging, leading to sustainable global health. It is suggested to promote and practice global health collaboration education, promotion activities among sectors to raising the health status of societies to accepting globalization and prosperous future societies.

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USING AN INNOVATIVE TELEHEALTH MODEL TO SUPPORT PROVIDERS IN GEOGRAPHICALLY DISPERSED AREAS WHO DELIVER CARE TO HIV-POSITIVE PREGNANT WOMEN

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Human Immunodeficiency Virus (HIV) remains a global pandemic, with mother-to-child transmission (MTCT) persisting in many areas due to inadequate access to early diagnosis and prenatal care. These barriers to HIV management are most pronounced in rural areas where resources are often limited, both in the United States and abroad. As many rural primary care providers do not receive a high volume of HIV patients, they often face challenges in maintaining familiarity with current HIV guidelines, including those related to MTCT. Frontier AETC ECHO is an innovative telehealth program that offers longitudinal teaching and mentorship as well as remote consultation to community HIV practitioners with the goal of strengthening capacity of the HIV workforce, supporting high-quality HIV care, and disseminating best practices in HIV medicine. This virtual peer-to-peer support network connects community HIV providers and a multidisciplinary team of specialists at the University of Washington across vast distances and employs real-time, case-based discussion and lectures to educate and support providers in low-resource and rural settings. Here, our goal is to assess the impact of the Frontier AETC ECHO program on provider management of HIV in pregnancy through two means: 1) reviewing cases of HIV in pregnancy presented by community practitioners to the ECHO network, and 2) a survey of community providers who regularly participate in ECHO. The survey assessed providers' knowledge, comfort level, and local resources for managing HIV in pregnancy. More than half of participants responded. All patient cases had the successful outcome of prevention of MTCT of HIV. Patient and provider-based outcomes support that ECHO is an efficient tool for supporting management of HIV in pregnancy, with providers reporting increased knowledge as a result of both presenting and observing cases on ECHO and many providers reporting they would be refer their patient to another provider if ECHO support were not available. These results demonstrate the potential of this unique model for application to other prevalent healthcare issues in rural and low-resource settings.

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COMMUNITY-BASED SCREENING FOR CARDIOVASCULAR RISK USING A NOVEL MOBILE HEALTH (MHEALTH) TECHNOLOGY IN RURAL KENYA

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An increasing burden of non-communicable diseases, including cardiovascular disease (CVD), in low- and middle-income countries demands innovative approaches. In remote, resource-constrained settings, models of laboratory-based, physician-supervised management are not sustainable. Community health workers (CHWs) can provide primary health care, yet many lack necessary background, training and skills. Communication technology, such as mobile health (mHealth) has the potential to augment CHW capacity. We hypothesized that mHealth could be used to identify individuals at high CVD risk in remote communities with poor access to health clinics who would benefit from education and pharmacologic interventions. We designed and implemented a novel mHealth tool based on principles and data from the WHO Package of Essential Noncommunicable Disease Interventions for Primary Health Care. Our "two-way" mobile phone application collects and centrally stores SMS

text message data entered by a CHW on a subject's age, gender, smoking, diabetes, and systolic blood pressure, and returns as SMS text message the category of 10-year CVD risk: "green" <10%; "yellow" 10 to 140mmHg. The prevalence of hypertension was similar in men and women (22/97 [23%] men vs 26/126 [20%] women, p=0.74). Only 2/223 (0.9%) of subjects reported a history of diabetes. The estimated 10-year risk of CVD event was <10% ("green") in 218/223 (98%) and 10-20% ("yellow") in 5/223 (2.2%). All subjects received immediate feedback on their risk profile, counseling on cessation of tobacco use, healthy diet and exercise, and referral to the a nearby clinic for follow-up for patients with elevated CVD risk. Acceptability of the mHealth application, and of CHW CVD risk screening by subjects was excellent. We have developed an mHealth tool that can be used by CHWs to screen for CVD risk factors, demonstrating proof-of-concept in rural Kenya.

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DOMESTIC IMPLEMENTATION OF THE INTERNATIONAL HEALTH REGULATIONS: BRINGING THE WORLD OF HEALTH SECURITY HOME

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Domestic Implementation of the International Health Regulations: Bringing the World of Global Health Security Home The IHR is an agreement between all WHO Member States, 196 countries, to strengthen public health preparedness and response capacities globally. Under the IHR member states are required to establish core capacities to detect, assess, report and respond to public health emergencies. These core capacities allow for the assessment and notification of public health emergencies with potential international impact to World Health Organization (WHO) through the international network of IHR NFPs. In the United States, the Division of International Health Security (DIHS) in the Office of the Assistant Secretary for Preparedness and Response (ASPR) coordinates, and is a critical component of, the three-part U.S. NFP. In this role, DIHS advises the federal government on international IHR policies, develops domestic polices and processes to address IHR obligations, coordinates IHR-related communications and technical exchanges with international partners and domestic stakeholders, and leads efforts on bilateral policy and capacity-building exchanges with foreign NFPs. State, local, tribal, and territorial (SLTT) public health professionals play an integral role in the fulfillment of U.S. obligations under the IHR Specifically, the US NFP works closely with federal departments and agencies who support SLTT reporting and response networks (e.g., CDC, FDA, DOI, USDA) to ensure communication of IHR-relevant events to WHO and international partners. Additionally, the US NFP uses SLTT self-assessments associated with the federal Public Health Emergency Preparedness cooperative agreement and the Hospital Preparedness Program to inform annual assessments of domestic IHR capacities and reporting to WHO. Attendees of this Learning Session will have the opportunity to discuss management of the US NFP, better understand the policies and processes central to the U.S. NFP, and consider how capacities at the state and local level support U.S. IHR obligations and, as a result, reinforce domestic and international health security.

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IMPLEMENTATION OF INTEGRATED POINT-OF-CARE TESTING FOR HIV SYPHILIS MALARIA AND ANAEMIA (IPOC) IN ANTENATAL CLINICS IN WESTERN KENYA

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Prevalence of HIV, syphilis, malaria, and anemia is high among pregnant women in western Kenya; testing for these is currently part of the country's antenatal recommendations. However, implementation of the full antenatal testing profile is limited because not all tests are done in smaller facilities, requiring women to be referred to distant labs. We conducted an implementation study to introduce point-of-care testing for HIV, syphilis, malaria and anemia (iPOC) in small rural health facilities in western Kenya. Seven small facilities without screening tests were purposively selected to introduce iPOC in December 2014. Antenatal nurses received a week of training. All 4 point-of-care tests were provided. For 8 months, 588 exit interviews were done to assess testing uptake and quality of care. Comparison of ANC register data before and after iPOC was done to assess uptake increase. On-site observations of testing for quality control were done at 3, 6, 9 and 14 months. To determine the perception of the ANC experience with iPOC, 12 focus group discussions were held among women who had attended ANC recently. In-depth interviews on iPOC delivery were also done with program stakeholders and 16 iPOC nurses. Overall implementation success was assessed using 5 indicators: program acceptability, adoption, appropriateness, feasibility and fidelity. We assessed the impact of iPOC on operations using discrete event simulation (DES) modelling to compare system outputs with and without iPOC. Over a 4-week period in August 2015, clients attending MCH services at these facilities were observed and their process times taken to construct flow pathways and parameters to input into the model using WITNESS software. DES model outputs will include time to complete testing, waiting times, duration of activities, staff and resource impacts, disease diagnosed, process bottlenecks, and cost impacts in clinics with and without iPOC intervention. This study will give insight into the implementation of iPOC from the client, provider and health system perspective for future program delivery. The study will be completed in August 2016 and results will be presented.

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CROWDSOURCE REPORTING OF INFECTIOUS DISEASES DURING A DISASTER: A NOVEL TECHNOLOGY APPROACH

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Crowdsourcing of patient text data related to infectious diseases such as viral respiratory is a viable option for reporting of cases in the event of a natural disaster such as floods and hurricanes. The challenge during a natural is that access to the Internet is limited and traditional websites for logging infectious reports may not be operational. The data gathered from this process will be kept confidential and used as planning data and tracking cases for healthcare provider resources in a community. A personal cloud server is a solution to host a data collection system that will crowd source infectious disease data for a community such as gender, age of patient, patient history and medications, etc. Community citizens, public employees, healthcare providers can input data to the wireless personal cloud infrastructure via a smartphone or a laptop. These personal cloud servers can be co-located in storm-protected buildings with a back-up power source and be connected to a wireless wide area network (WAN). Even in the absence of general Internet connectivity, a user just has to be in the range proximity of the wireless WAN and be able to

upload their text data to the personal cloud server. After regular Internet is restored to the community, an administrator can download the text data from the personal cloud server to a larger database for cartographic and statistical analysis. The outcome of this project is to identify the user and technical requirements and human interface & security challenges for connecting several connecting personal cloud servers to a larger cloud-based database and creating a strategy for implementing, testing and evaluating this process in a real-world natural or man-made disaster. In onclusion, local healthcare administrators will find this system a viable alternative to reporting of infectious diseases during a natural disaster. The continuous reporting of infectious diseases at ground zero level is important for planning, monitoring and tracking of these cases. This novel approach allows for the uninterrupted collection of infectious disease text data for later analysis.

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EBOLA VIRUS DISEASE PREPAREDNESS AND RESPONSE STRATEGIES IN ETHIOPIA AND LESSONS LEARNED FROM WEST AFRICAN COUNTRIES

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Ebola Virus Disease Preparedness And Response Strategies In Ethiopia Lessons Learned From West African Countries Ebola virus disease has claimed more than 11,000 lives during the current epidemic in West Africa making it the deadliest outbreak since Ebola was discovered in 1976.On 8 August the World Health Organization declared Ebola outbreak as a public health emergency of global Concern. The Ebola outbreak in West Africa is unprecedented in terms of its geographical scope. It began in Guinea in early 2014 and quickly spread to the neighboring countries. Inadequate health infrastructure and overall fragile health system has fueled rapid spread of the outbreak in West Africa. The severity of the outbreak is exacerbated by lack of understanding about the disease by communities and lack of experience among health-care workers .Lack of adequate treatment facilities, rumor, fear and stigma has in turn led families to keep sick patients at home, risking further spread of the virus. Resistance to proposed response measures as well as traditional burial practices further aggravated high transmission of the outbreak with devastating economic and livelihood implications. Ethiopia by the virtue of its geographic position and being political capital of Africa and being a major transport hub in the east Africa region is very prone to the deadly virus due to importation from Ebola riddled countries. Hence it has swiftly enhanced its domestic preparedness to respond in a predictable manner in the event of Ebola outbreak .It has employed state of the art screening measures at main entry ports and has also been engaged in fighting the outbreak at its source by sending medical teams to Ebola-stricken nations. This paper reviews the current Ebola preparedness and response strategies in Ethiopia, challenges and crucial lessons learned from West African countries and map out the strategies that are in place for improvement preventing future outbreaks.

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ASSESSMENT ON COMMUNITY BASED HEALTH INSURANCE IMPLEMENTATION IN RURAL ETHIOPIA

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Ethiopia is among developing country with more than 90 million populations. The country is highly depending on external development partner to fiancé the health need of this huge population.Since the presence of donors' resource is unpredictable, inadequate and unsustainable, the health sector is doing its best to bring sustainable domestic financing through implementing health care financing reforms (HFR). Community based health insurance (CBHI) is one of the reforms under implementation at piolet level in rural part of the country. This research is aimed to assess the implementation of CBHI and understand dropout, enrollment, re-enrollment, regional disparities and service uptake to inform the scale up strategy of the program. Both quantitative and qualitative methodology used to generate evidences. Around 1600 household surveyed in the piolet and control districts. Four rounds of survey data from same households, with same survey instrument and at the same season applied to understand the trends of key CBHI implementation indicators aforementioned above with econometrics model. It is found that the uptake of the scheme has been 41% of the target households in 2012 and this has increased to 58% in 2015. Membership renewal is more than 80% of the initially enrolled households. The Ethiopian scheme enrollment and retention rates are impressive as compared to the experiences of other African countries. In terms of uptake, there are substantial differences across the pilot regions. It is found that Amhara is the best preforming region with coverage rate of 68% while Tigray is the lowest one with 49% uptake rate. Variations in the extent of ownership and commitment from local administration bodies to concerted mobilization effort during the defined renewal time frame, waiting time, renewal timing, and allocation of targeted subsidies for indigent groups contributed to the differences in the coverage of the scheme across the pilot regions. Drop out decision does not significantly relate with socioeconomic status.

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WEAK COLD CHAIN CAPACITY CAN COMPROMISE IPV INTRODUCTION IN SUB-SAHARAN AFRICA: A CASE STUDY FROM CAMEROON

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In 1988, the World Health Assembly resolved to eradicate poliomyelitis by the year 2000. Since then, tremendous strides have been made towards this goal and the incidence of polio has fallen by more than 99%. As part of the endgame strategy, the World Health Organization recommended countries to introduced at least one dose of inactivated polio vaccine (IPV) by 2015 into their routine immunization schedules. However, there is emerging evidence that weaknesses in cold chain systems can hamper successful introduction and roll out of IPV in low income countries. In Cameroon, for instance, over half of country's stock was damaged prior to IPV launch. In this paper, we explored the reasons that led to this loss and the potential implications. We collected data from central, regional, district and health facility levels on IPV stock status, functional status of cold chain equipment, temperature monitoring practices and IPV coverage rates. Following the decision to introduce IPV, Cameroon imported a total of 840,000 doses of IPV. Nearly 500.000 of these doses were damaged at a central level. An additional 18,000 doses were damaged in one region. The primary causes for this loss were dysfunctional cold chain equipments, limited staff training and weak temperature control practices amongst others. The estimated financial loss was US$452,000. This loss, alongside challenges in importing additional doses because of limited global production capacity, resulted into prolonged stock out in many health facilities, which turn affected national coverage rates. With limited global IPV production capacity, weak cold chain systems can affect the availability and adequacy of IPV for routine immunization. This, in turn, may affect IPV coverage rates and may ultimately compromise polio eradication efforts.

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COMMUNITY HEALTH VOLUNTEERS' USE OF MOBILE PHONES TO REPORT MASS DRUG ADMINISTRATION (MDA) DATA

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There is growing evidence of the potential of mobile phones to support public health programmes. Community health volunteers (CHV) who play a major role in mass drug administration (MDA) for Neglected Tropical Diseases Control Programmes (NTDP) are being considered as potential aides in improving data reporting from the community level. Little is known about technology adoption for MDA reporting by the CHVs working with NTD programmes. This study sought to determine CHVs willingness to use mobile phones to report MDA data and factors associated with their intention to use. This was a mixed methods study with data collected during two consecutive annual LF MDAs in two districts in Ghana. A structured questionnaire was used to collect data on socio-demographic characteristics, mobile phone use experience and readiness to adopt mobile phones for MDA data reporting. Readiness was measured using the Unified Theory of Acceptance and Use of Technology model. CHVs were trained to submit summarised MDA reports by SMS in the first year in both districts and USSD in year two in one district. Overall mobile phone ownership was 99% while 33% had text messaging experience. About 31% ($\chi 2=2.67$, $p<0.05$) submitted their MDA report using the mobile phone. The model explained very little about the CHVs behavioural intention (R2= 0.17). Willingness to submit text was high however actual submission was low. Factors associated with the low response were the CHVs perception of effort needed to send the text, social influence, poor network quality and texting experience. Factors such as age and gender did not have significant effect on their intention to use and actual use. General enthusiasm for MDAs should be reignited among CHVs. National policies on network quality improvement will provide enabling environment for implementation of such technology.

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COST-EFFECTIVENESS OF DENGUE VACCINATION IN FIVE SOUTHEAST ASIAN COUNTRIES

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In 2015, the first dengue vaccine was licensed in several endemic countries, initiating a valuable new control tool. Decisions about its use in public programs depend on anticipated health benefits, costs, and cost-effectiveness. To inform policy discussions in Asia, we used a transmission model calibrated with data collected during vaccine efficacy Phase III trials. Costs of vaccine administration, procurement, and dengue treatment were based on publications and reports. Each vaccine dose was projected to cost $2 for vaccine delivery plus $20 for vaccine procurement. Our base case assumed that a 3-dose vaccination program would be offered to all 9 year-old children each year, plus a 4-cohort initial catch up (10-13 year-olds), phased over 3 years and achieving 80% coverage. Our base case expressed costs in 2013 US dollars from a health system perspective, conducted 100 simulations with a 30-year horizon to account for variability in dengue transmission and uncertainty on vaccine efficacy, measured health impacts in disability-adjusted life years (DALYs), and assessed cost effectiveness as $/DALY averted. Our base case results indicated that vaccination would save from $0.11 (Vietnam) to $1.72 (Malaysia) in annual per capita dengue treatment costs and would reduce dengue-related DALYs by 26% (Thailand) to 32% (Malaysia). Cost effectiveness ratios, expressed as multiples of each country's per capita gross domestic product (GDP), were: Indonesia (0.17), Malaysia (-0.13), Philippines (0.56), Thailand (0.20), and Vietnam (3.57). In Malaysia, the vaccine is cost saving. Using WHO benchmarks of 1 and 3 times per capita GDP, the vaccine is highly cost-effective in Indonesia, Philippines, and Thailand (being below the most stringent benchmark), but is not cost-effective in Vietnam from a health system perspective. Cost effectiveness results were similar for other vaccination programs (0 to 8 catch-up cohorts) and coverage rates (50% to 80%). The consideration of a societal perspective, increasing dengue incidence, dengue's adverse impacts on tourism, and rising real incomes and health care costs further favor the case for vaccination.

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COMMUNITY PERCEPTIONS OF HYPERTENSION IN LOW-RESOURCE SETTINGS IN THE DOMINICAN REPUBLIC

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The bateys (sugar cane towns) of La Romana, Dominican Republic are home to a large number of Haitian migrant workers. Due to their poor living conditions and barriers to accessing care, the health status of batey residents is severely compromised. Although efforts have been made to improve the health outcomes of batey residents, the discontinuity and lack of culturally appropriate care significantly reduces the effectiveness of these efforts. This study describes the perceptions of hypertension (HTN) among batey residents, to aid in developing a protocol that improves quality of care provided in the bateys. The goal of this study was to assess participants knowledge of HTN and HTN care. Data was collected using surveys administered verbally (N=81) using an interpreter fluent in Creole and Spanish. Of those surveyed, 54 were female and 27 were male, with 59 participants aged 40 or older. Results showed that batey residents had learned about HTN from various sources including local clinics, hospitals, foreign medical teams and other batey residents. Headache (n=18), dizziness (n=15), shortness of breath (n=10), and weakness (n=7) were commonly reported symptoms of HTN. Additionally, most respondents were aware of the nearest clinic, with a majority (n=62) accessing clinics by taxi. Finally, batey residents viewed financial cost (n=24), a lack of clinicians in the bateys (n=20), and underdeveloped transportation (n=10) as the most significant barriers to accessing HTN care. Our findings reveal that males are underserved by existing models of HTN care, as many work during the day when mobile clinics visit the batey. Results also suggest that an optimized protocol should focus on bringing care closer to the bateys to increase access. Finally, there is a need for coordinated education and treatment programs between the different care providers in batey communities to ensure sustainable and continuous care. Overall, these results shed insight on how chronic disease protocols can be successfully implemented in low-resource settings to improve health outcomes.

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PEACE AND POST-CONFLICT HEALTH: NEONATAL AND MATERNAL MORTALITY AFTER CIVIL WARS WITH DIFFERENT ENDS

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The outbreak of "peace" after civil war is characterized by deeply entrenched grievances, mistrust, and insecurity. Violence often persists at high levels as the beneficiaries of resilient war economies shift to alternative means of maintaining profit and power. Institutional violence disproportionately affects different segments of society as the quality of peace may be dictated by a conquering victor who may seek retribution or well-armed warlords invited to the negotiating table whose

interests may only be the legitimization of territorial and economic gains achieved through battle. This study tests the assumption that diplomatic negotiations at the end of war protect the health and interests of vulnerable, non-combatant populations such as women and children. We examined neonatal and maternal health in societies after civil war whereby neonatal mortality rates and maternal mortality ratios are compared between three peace types: a peace imposed by the victor, a negotiated peace between capable warring parties, and no peace/continued war. The Uppsala Conflict Data Program Armed Conflict Dataset was queried with UNDP neonatal mortality rates (NMR) and maternal mortality ratios (MMR) for five years after conflict termination in Africa, South Asia, and South America between 1990 and 2013. Health data were annualized to net change year-on-year relative to health at the point of peace declaration. Health outcomes by peace type were compared by Mann-Whitney U-tests at α=0.05 with 95% confidence intervals. We found that NMR fell by 1.8 and 1.9 deaths per 1,000 births in VP and NP, respectively, with no statistical differences observed; we found that MMR fell by 35.5 and 55.6 deaths per 100,000 births in VP and NP, respectively, with no statistical difference observed. Although a statistical difference could not be appreciated at five years, concerning trends in MMR between different peace outcomes were observed and suggest a difference in the quality of peace for women.

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MANAGEMENT OF DENGUE HOSPITALIZATIONS IN BRAZIL DURING AND OUTSIDE EPIDEMIC PERIODS: INSIGHTS FROM DATA MINING

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Brazil reported 1.5 million dengue cases in 2015, more than any other country. Most of the Brazilian population rely exclusively on the publicly funded health care system SUS (Sistema Unico de Saude). All hospitalizations paid for by SUS are registered in a publicly available database (SIH/SUS). We used the information included in this database to assess the implication of the epidemic nature of dengue on the management of dengue hospitalisations during and outside epidemic periods. Over 2008-2015, the SIH/SUS database describes 92 million admissions in 15,002 departments of 4,293 hospitals, of which 354k are admissions associated to a dengue diagnostic. For the analysis, hospital departments were classified as "High Dengue Activity" (HDA) departments or "Low Dengue Activity" (LDA) departments using the following criteria for HDA departments: at least 200 dengue admissions over 2009-2015 and a maximum dengue patient load (daily bed count) exceeding 20% of the estimated bed capacity of the department. Over 2009-2015, 43% of admissions occurred in 274 HDA departments, while the remaining 57% dengue cases were managed in 4,070 LDA departments. The maximum level of dengue patient load in the 274 HDA departments was notably high, at 43% on average and up to 62%. The overall patient load indicated that these HDA departments operate near or even sometimes beyond their maximum capacity during dengue epidemic periods. The systematic analysis of admissions to HDA departments using data mining tools also led to the identification of subgroups showing an excess of mortality during dengue epidemic periods. These subgroups are associated to dengue-related diseases and case management characteristics. We also observed a larger proportion of cases managed in LDA departments during dengue epidemic periods. Finally, we observed a higher dengue case fatality rate in LDA departments than in HDA departments (0.9% and 0.4% respectively; OR=2.1 (1.9-2.3)).This analysis highlights the consequences of the seasonal aspect of dengue on the management of dengue hospitalization in Brazil and some implications for dengue-related mortality.

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BALANCING RISK AND COSTS IN CONTROLLING THE INTERNATIONAL SPREAD OF INFECTIOUS DISEASE OUTBREAKS WITH ACTIVE MONITORING

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The 2014-2015 west African Ebola outbreak was an unprecedented global public health emergency that underscored the ease with which pathogens can spread in today's interconnected world. To improve rapid identification and evaluation of individuals at risk for Ebola, some countries actively monitored of individuals who were returning from Ebola-affected regions. A common policy was to have individuals under active monitoring make daily contact with local health authorities each day for 21 days after their last potential exposure. Active monitoring has shown promise as a tool in preventing and responding to outbreaks of pathogens that pose a grave threat to public health. It may also play an important role in containing outbreaks of emerging pathogens that could rapidly spread throughout a population. We developed a framework for evaluating the risks and costs associated with active monitoring using. As a case study, we analyzed new data on the cost of the Ebola response in New York City and existing data on the incubation periods of Ebola, MERS-CoV, and smallpox. Our analysis provides empirical evidence that could inform the design and implementation of future active monitoring programs. For example, to tolerate the level of absolute risk implied by Ebola active monitoring programs, our model would suggest that for both MERS-CoV or Smallpox, a duration of active monitoring could be set at less than 2 times the median incubation period for each disease. Furthermore, for low-risk and high-risk individuals, our model provides estimates of how long individuals should be monitored to minimize the expected cost of the monitoring program. This model could be used to help guide future assessments and data collection on active monitoring programs. It also provides a pathway for data to inform the design of active monitoring programs for the next global epidemic.

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UNDERSTANDING RESEARCH ACTIVITY IN THE HEALTH SECTOR OF UZBEKISTAN: IMPLICATIONS FOR HEALTH RESEARCH CAPACITY STRENGTHENING

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Health research capacity building between high income countries and low income countries is being recognized as an important field in achieving health equity. Evaluating the research capacity is considered to be one of the key principles in achieving good practices in research capacity building. This study sheds light on the current research capacity in health sector of Uzbekistan. Bibliometric analysis was applied to examine the publications trends in health research to map the Uzbekistan research production along with the key research topics. A search strategy was built to retrieve journal articles from the Web of Science (WOS) and PubMed Medline from 1991-2015. A total of 430 articles were identified and 321 articles were analyzed after exclusion criteria were applied. The number of articles trended upward with accentuated growth during 2000 to 2010 of 180 articles, six times than the earlier decade (1991-2000). 62.7% of the articles were related to communicable diseases, newborn, maternal, and nutritional causes and 26.8% of the articles reported on non-communicable diseases while only 9.5% of articles reported research on health systems. In total, 60.4% of papers involved international collaborations with institutions in USA(26.8%), Japan(13.4%), and Russia (10.3%) followed by multilateral organizations such as WHO and MSF. Uzbek authors in international collaborations published in high impact

factor journals (average of 2.46) while studies reporting of only Uzbek authors published in low impact factor journals (average of 0.205). Health research output in Uzbekistan has increased since independence but Uzbek authors face difficulty in making their research visible in international community without international collaboration.

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RESIDENT-DRIVEN TELEDERMATOLOGY IN HAITI: A SYMBIOTIC PARTNERSHIP IN DERMATOLOGIC EDUCATION AND HEALTH

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It is important for residents to pursue relevant exposures during training for their intended career niche. I created a resident-driven teledermatology program in Haiti with the dual-goal of providing care to patients lacking access while broadening resident exposure in a tropical underserved population. 59% of Haitians live on less than $2 a day with a life expectancy of 63 years. Up to 20% of outpatients in the tropics have a skin complaint. Infrastructure and health services are limited in Haiti, but internet access and cellular service is present throughout the country-making teledermatology a more practical approach to see patients. The foundation for this program was built upon multiple medical aid trips to Haiti working alongside local Haitian Dermatologists. The partnerships developed with local practitioners helped to create the foundation for this program through patient evaluation, referrals, follow up, and treatment. A donated used cameraphone held by a local nurse offers a portable method to take clinical images which can be emailed at any time ("store and forward telemedicine")- ideal in an area with sporadic internet connectivity. A patient questionnaire provides relevant information without the time & cost of a physician visit. Recommendations are made within 48 hours by volunteer residents, overseen by attending physicians, and conveyed to local health providers. Donations cover the costs of labs, biopsies, or medications for patients who cannot afford them. Referrals are provided to those requiring additional care in-person. From mycetomas to varicella and an increased rate of genodermatoses (due to founder effect in an island population), the educational value for residents is vast. Dengue and Chikungunya viruses appeared in the Caribbean a full year before cases appeared in Florida and as we anticipate the spread of Zika virus our residents have already become clinically familiar through Haiti. Establishing a telemedicine program is an inexpensive, reproducible and feasible endeavor for residents seeking niche experience in the field of global medicine while providing healthcare to those most in need.

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AN INNOVATIVE TRAVEL MEDICINE APPROACH USING MHEALTH TECHNOLOGY TO DESCRIBE HEALTH RISKS TO TRAVELERS

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Emerging mHealth technology shows great potential in more accurately and completely documenting travel itineraries and modeling health and disease risk patterns of travelers, including accidents/injuries, infectious and other non-communicable disease outcomes. Our study aims to address several major shortcomings in travel health research by using a smartphone application to collect detailed information on health behaviors, symptoms and accidents, and environmental risk factors during travel. In partnership with the Swiss Federal Institute of Technology (ETH) Wearable Computing Lab, the Epidemiology, Biostatistics, and Prevention Institute at the University of Zürich have developed a novel data collection instrument and analysis concept: a smartphone application that collects data on 1) travelers' exact itinerary and environmental conditions using passive GPS localization, and 2) a daily-self report questionnaire on health risk behavior, accidents, and symptoms while traveling. A prospective cohort of 107 travelers planning travel to Thailand between January and June 2015 was recruited from the travel clinics of Zurich and Basel in Switzerland. Of the 101 recruited travelers that went to Thailand, 75 (74.3%) answered at least 1 questionnaire during travel, 10 (9.9%) had technical difficulties, and 16 (15.8%) dropped out or were lost to follow-up. Travelers filled out a median of 12.0 surveys during their trip (range: 1-30), corresponding to a median completion rate of 85.0% days of travel. Questionnaire completion rates were best for shorter trips, with a survey completed on average 94.8% of days for trips less than 10 days. Non-infectious disease health outcomes were common, with 22.7% of travelers experiencing an accident, 49.3% mental distress, and 14.7% an animal bite. Use of a smartphone application to collect health information is technically feasible and acceptable among the traveler population, minimizes recall bias, and greatly increases the quality and quantity of data collected during travel. MHealth technology shows great potential for innovation in the field of travel medicine.

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ASSOCIATION BETWEEN IMPROVED HOUSING CHARACTERISTICS AND MALARIA PREVALENCE IN CHILDREN UNDER FIVE

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In the past decade, malaria vector control strategies in sub-Saharan Africa have focused on the use of insecticide-treated nets (ITNs) and indoor residual spraying (IRS) in combatting malaria. House construction using modern, impermeable materials also improves vector control. Past studies have examined the association between household characteristics and malaria through meta-analysis or localized surveys; however, there has been few studies using nationally-representative population-based surveys. This study examined 24 Demographic Health Surveys (DHS) and Malaria Indicator Surveys (MIS) with data on malaria parasitemia status in children under five and household characteristics such as type of flooring, wall, and roofing materials. Logistic regression was used to assess whether improved flooring, wall, and roofing types were protective against malaria after controlling for ITN use, IRS spraying in the past 12 months, household wealth status, age of child, sex, and malaria endemicity in survey specific analyses as well as meta-analysis. Results of the country-specific analyses showed a significant protective effect of an improved roof on malaria infection in children in 10 of the 24 surveys included (from Benin, Burundi, Cameroon, Mali, Malawi, Nigeria, Senegal, Tanzania and Uganda). Results of the meta-analysis show a protective effect of improved roof (metal, wood, ceramic tiles, cement, and shingles) on malaria infection in children under five (OR=0.82; 95% CI: 0.74-0.92). Marginally significant protective effects of improved walls (baked bricks or cement blocks) and improved floor (not earth, sand or dung) on malaria infection in children under five was also found (OR = 0.91; 95% CI: 0.82-1.00) and (OR=0.95; 95% CI: 0.86-1.04, respectively). Results corroborate findings from other studies that show housing as an important risk factor for malaria. Findings suggest that investments in improved housing may contribute to sustainable development goals by conferring protection against malaria as well as other socio-economic benefits.

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THE IMPROVING MALARIA CARE (IMC) PROJECT'S CONTRIBUTION TO FOLLOW UP A PILOT TO USE RAPID DIAGNOSTIC TESTS (RDTS) AT THE COMMUNITY LEVEL IN BURKINA FASO

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Early and correct case management of malaria in health facilities and at the community level is among the priorities of Burkina Faso's National Malaria Control Program (NMCP). In line with this initiative, the NMCP piloted use of Rapid Diagnostic Tests (RDTs) by Community Health Workers (CHWs) to confirm malaria cases in the three health districts of Kaya, Saponé and Nouna between 2013 and 2015. With PMI support, follow-up visits were organized to document best practices, as well as challenges, on RDT use by CHWs that could serve as lessons learned for scale-up. During follow-up visits, malaria commodities management (supply, storage and use) at the community level was examined, use of RDTs was assessed, and implementation at the community level was discussed with all actors at regional, district, health facilities, and community levels. The team examined the monitoring/supervision processes at all levels, used a check list on malaria commodities management, and employed a questionnaire for each type of actor. Both qualitative and quantitative data have been collected. A total of 108 persons were contacted including 32 CHWs, 42 community leaders and 34 health care providers and managers. Findings revealed frequent stock-outs of RDTs and artemisinin-based combination therapies, non-payment of stipends to CHWs (a demotivator) and insufficient supervision of CHW by health teams. From the community perspective, 66% of community leaders were satisfied with their CHW's work (diagnosis and treatment of uncomplicated malaria and referral of severe cases to health facilities). However, 46% of community leaders complained of frequent stock-outs and unanimously agreed on the importance of regular payment of premiums to CHW. Follow up of the pilot was valuable in obtaining community, CHW and health worker perspectives for improving the program. While the community finds the program acceptable, its sustainability will require that solutions be found for stock-outs, non-payment, and insufficient supervision before scale up takes place.

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AVAILABILITY OF ORS, ZINC, AND AMOXICILLIN AMONGST PUBLIC FRONTLINE WORKERS IN UTTAR PRADESH, INDIA: A KEY DETERMINANT TO REDUCING UNDER FIVE MORTALITY DUE TO CHILDHOOD DIARRHEA AND PNEUMONIA

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In India, diarrhea and pneumonia contribute to around 12% and 23% of all under five deaths. Under the National Rural Health Mission, Government of India's flagship initiative to provide quality health care to rural population, accredited social health activists (ASHAs), the community-based workers, have been key players for the provision of health services in Indian villages. ASHAs have received a number of trainings in the past to strengthen their skills in the management of childhood illnesses. Despite the trainings and widespread presence of ASHAs, the decline in the mortality rates due to childhood diarrhea and pneumonia has been slow. 473 ASHAs sampled as per a predetermined randomization protocol were interviewed face-to-face from six districts of Uttar Pradesh to understand their treatment recommendation practices and proportion of diarrhea and pneumonia cases seen by them. Additionally, a medicine audit was undertaken to check the availability of ORS, zinc, and amoxicillin. On average, ASHAs assessed less than one case of diarrhea and pneumonia in the last 7 days. 72% recommended both ORS and zinc, 16% ORS alone, and 0.2% zinc alone, and none recommended amoxicillin. Of those ASHAs that did not recommend ORS and zinc, non-availability was mentioned as the main reason for non-recommendation. During the medicine audit, 43% and 17% had a stock of ORS and zinc respectively, and none had amoxicillin. Significantly low number of diarrhea and pneumonia cases seen by ASHAs means that there is little opportunity for them to consolidate and refine their skills. Also, a high proportion of ASHAs recommended ORS and zinc for diarrhea, but did not have ORS or zinc in their drug kits. Lack of amoxicillin in their drug kits was a significant factor in not recommending amoxicillin for pneumonia. The success of government rural health programs is dependent on ASHAs and their ability to cater to the health needs of the rural population. Ensuring adequate stocks of ORS, zinc, and amoxicillin with ASHAs is crucial to accelerating the decline in diarrhea and pneumonia mortality rates.

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COMPREHENSION OF SURGICAL INFORMED CONSENT IN HAITI

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Informed consent has long been considered an essential requirement of surgical care in the United States. However, little is known about the use of informed consent on international surgical trips. Since 2008, a multi-disciplinary team from Emory University has partnered with Hospital St. Therese in Hinche, Haiti to provide surgical care. An informed consent tool has been developed and evaluated by the Emory team to prepare patients for surgery. All patients at Hospital St. Therese scheduled for surgery by the Emory team (n=52) received a dual video and written informed consent both translated into Creole describing the procedures, risk and benefits of both surgery and anesthesia. Procedures performed were primarily inguinal herniorraphy and open prostatectomy. Following the informed consent, patients completed a survey translated into Creole evaluating their understanding of and satisfaction with the procedures using a tablet app both before (n=48) and after surgery(n=47). Prior to surgery, 91% of patients were able to correctly identify their surgical procedure. The majority of patients were able to identify the most common risks of surgery including pain (85%), bleeding (80%) and infection (70%). 98% of patients were satisfied with the informed consent process and 91% of patients would have their operation again at discharge. Our survey revealed that our ability to obtain informed consent was limited by language barriers despite the use of translators (61%) and poor literacy (54%). We plan to refine our informed consent process to better address these challenges in the future. The results of our survey demonstrate that an informed consent tool can aid in preparing patients for surgery but that communication barriers inherent to the setting of international surgical trips should be considered in the development of successful informed consent tools.

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LONG-LEAD EL NIÑO FORECAST INFORMATION TO SUPPORT PUBLIC HEALTH DECISION MAKING: APPLICATION TO DENGUE EPIDEMICS IN ECUADOR

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El Niño Southern Oscillation (ENSO) is a high-impact climatic phenomenon causing substantial changes in the weather worldwide. It leads to floods or droughts in certain regions of the globe, damaging agriculture and marine ecosystems, and increasing the risk of infectious disease epidemics such as dengue epidemics in some tropical countries. Therefore, ENSO forecasts could help authorities to plan in advance of imminent disasters, to mitigate the risk, and to protect vulnerable communities. A structural time series model, which uses a state space approach and explanatory variables relevant to the El Niño (EN) evolution, has been developed and tested to predict sea surface temperature (SST) in the equatorial Pacific Ocean (in the Niño 3.4 region). The model configuration is specifically tailored to forecast EN at long lead times of 24 months or more, going well beyond the traditional ¨spring barrier¨ of ENSO prediction. The forecasting scheme provides information about the amplitude of the events, their duration, and the peak time of the SST. This information could be used to support decision making, especially in tropical and subtropical countries, which are directly and severely affected by the anomalous temperature and precipitation rates that occur during and after El Niño events. Certain diseases are particularly sensitive to climate extremes. For example, a previous study found that the timing and magnitude of dengue outbreaks in El Oro province in Ecuador were associated with El Niño events. In this study, long-lead forecasts of equatorial Pacific SST (i.e. the Niño 3.4 index) are used within a dengue prediction model, to assess the extent to which dengue epidemics can be predicted well in advance. The ENSO forecasting model could have helped to predict the dengue epidemic that occurred in the region in 2010 as early as 30 months ahead. Thus, long-lead ENSO forecasts could be incorporated into dengue prediction models, to enhance the development of a dengue warning system for Ecuador and other tropical and subtropical countries sensitive to the ENSO phenomenon.

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DUAL PERSPECTIVES ON STIGMA: REPORTS OF EXPERIENCED AND ENACTED STIGMA BY THOSE AFFECTED AND UNAFFECTED BY PODOCONIOSIS

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Disease-related stigma is a public health concern steadily gaining global attention. Evidence consistently shows that an individual's attribution of disease cause can prompt or justify interpersonal stigma. However, few studies have explored causal beliefs about inherited disease and their influence on stigmatizing behaviours in low and middle income countries (LMICs). The study was conducted in 2013, in six communities in Wolaita zone, southern Ethiopia. A total of 1800 respondents (600 affected and 1200 unaffected parents of an index child aged between 3 and 6 years) took part in the study. Two versions of the enumerator-administered survey were created, with measures assessed in parallel on "experienced" stigma for the affected and "enacted" stigma for unaffected household respondents. Mean levels of reported enacted stigma were slightly lower (2.0, SD 0.7) than experienced stigma reported by affected respondents (2.2,SD 1.1). Males consistently reported significantly lower levels of experienced and enacted stigma than females, $p<0.0001$. Beliefs that podoconiosis was hereditary were significantly and positively associated with reported levels of experienced stigma among affected respondents and enacted stigma for unaffected respondents ($p<0.001$). There was no association between levels of stigma experienced by affected households with corresponding levels of enacted stigma reported by the neighbouring unaffected households. In conclusion, if stigma reduction interventions are to be successful, culturally-tailored, gender inclusive and innovative health education programs are required, directed at the general community as well as at patients.

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DOES HAVING HEALTH INSURANCE RELATE TO MEDICATION USE FOR HYPERTENSION IN THE DOMINICAN REPUBLIC?

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Hypertension, a major contributing factor to mortality worldwide, can be reduced by the use of antihypertensive medications. Studies within high-income countries have found that having health insurance increases the odds of use of medication among those with hypertension. However, there has been little examination of influence of health insurance on hypertension management in low and middle-income countries (LMIC) where hypertension affects a larger number of people. This study examined one LMIC, the Dominican Republic (DR), which has high rates of hypertension and a population incompletely covered by health insurance. This study investigated the relationship between having health insurance and recent use of medication among those diagnosed with hypertension using data from the 2013 Demographic and Health Survey (DHS) for the DR. Among survey participants who had been told by a health professional that they had hypertension, 42.3% of men and 42.7% of women reported taking antihypertensive medication in the two weeks preceding the survey. On bivariate analysis, women, but not men, were more likely to report having taken medication if they had health insurance. Within a multivariate model, having health insurance significantly increased the odds of medication use. Being a women and older age also increased the odds of medication use in the model. A sex by health insurance coverage interaction term was not significant. The wealth indicator demonstrated a more complex relationship with those in households classified in the poorest quintiles having higher medication use than those in the poorer quintile; this despite lower insurance coverage for the poorest. Perhaps non-insurance based targeted intervention efforts for the poorest may have extended coverage. This study is limited secondary to the truncated age range for women (15-49 years) available from the DHS. Future research should investigate types and quality of care provided for persons with hypertension for a fuller age range, with consideration of both health insurance coverage and wealth strata.

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LOW COST, IMAGING BASED DEVICE FOR PERFORMING A WHITE BLOOD CELL COUNT AND 3-PART DIFFERENTIAL AT THE POINT OF CARE

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The white blood cell (WBC) count and differential is an important laboratory diagnostic test. However, current methods for performing a WBC count and differential have high associated costs and infrastructure requirements and are therefore not available in low resource settings. There is a particular need for affordable tools to rapidly measure the WBC count and differential at the point of care in such settings. To meet this need, we have developed a portable device and low cost disposable cartridge that can be used at the point of care to perform a WBC count

and 3-part differential using 10 uL of blood obtained from a finger prick. The device is battery powered, can be deployed at the bedside, and produces results in under 5 minutes. The cartridge contains a microfluidic channel in which acridine orange is pre-dried. Blood from a finger stick is drawn into the channel via capillarity and WBCs are fluorescently stained with acridine orange, staining nuclei green and granules red without any sample processing. An all plastic fluorescence microscope is housed within the device, and a series of three images is captured and automatically analyzed to report the WBC count and the percentages of granulocytes, monocytes, and lymphocytes to the user. This is achieved by a novel image analysis program that identifies the WBCs in each field of view and classifies them into the WBC subtypes based on the red and green pixel intensity within each cell. The device has been recently tested with finger prick samples from 91 oncology patients at Lyndon B. Johnson Hospital in Houston, TX with WBC counts ranging from $2.7x10^3$ to $30.4x10^3$ WBCs/uL. Preliminary analysis of the results shows a strong linear correlation between the number of WBCs in the image field of view and the WBC count (R-squared 0.96). Further, the differential data also correlates strongly with the true values with an overall R-squared value of 0.92. We estimate at production scale the device can be developed for under $800 and the cartridge for less than $0.25, increasing access to this important diagnostic measurement to settings that currently cannot determine a patient's WBC count and differential.

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ADAPTING AND ENHANCING MALARIA INFORMATION SYSTEMS IN COUNTRIES ENTERING PRE-ELIMINATION

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There has been substantial progress in addressing sub-Saharan Africa's malaria burden. As countries reduce transmission and move from malaria control to pre-elimination, strong health management and information systems (HMIS) become critical to provide routine data to monitor progress, identify rebounds, and tailor new approaches for residual foci transmission. To ensure HMIS in sub-Saharan Africa are prepared to perform these roles in countries with the potential to move to pre-elimination, MEASURE Evaluation conducted a study to systematically measure the functionality of HMIS and to identify supporting factors. The study comprised a systematic literature review of HMIS performance assessments with a special focus on malaria. From an initial 1,581 peer-reviewed articles on HMIS in Africa, information was extracted and synthesized from a final 25 along the following subthemes: indicators of data quality, elements of well-functioning HMIS, supportive context for HMIS, and comprehensive measures of HMIS performance. The literature review revealed no uniform approach for assessing and improving the performance of HMIS and little guidance on how to adapt and enhance HMIS to keep them functional for countries at various points on the malaria control-to-elimination continuum. HMIS strengthening approaches generally focused on human capacity and participation, data quality and completeness, and a country-led enabling environment for policy and planning. The results of this desk review will be supplemented with in-country work to develop country case studies and a toolbox to help countries entering pre-elimination learn from the experiences of countries further along the continuum to adapt and strengthen routine data capture.

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THE PRIORITY REVIEW VOUCHER AS A MEANS OF ADDRESSING GLOBAL HEALTH PRIORITIES

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Neglected tropical diseases (NTDs) remain important sources of morbidity, mortality and disadvantage in low and middle-income countries, and yet the development and registration of new treatments for these diseases is rare. Funding to support the research and development of new medicines for these diseases is directly correlated with their low potential for return on investment, and remains insufficient to consistently meet regulatory standards. To drive this drug development, in 2007 the United States Congress created the priority review voucher (PRV) to reward sponsors who successfully register medicines to treat specified NTDs. The PRV is saleable to any company wishing to gain priority review of their new treatment, regardless of indication, creating a market that has yielded up to US$350 million per PRV. Although three new NTD treatments have been approved under the scheme, concerns have been expressed that sponsors benefitting from PRV sale are not bound to ensure treatment access and that the program does little to drive new NTD drug development. Medicines Development for Global Health (MDGH), a non-profit biotechnology company, partnered with the Global Health Investment Fund (GHIF), a U.S.-based social impact investment fund, to develop and register moxidectin for the treatment of onchocerciasis. MDGH is the first company to attract venture capital investment for a new treatment for a NTD on the basis of the PRV program. With limited commercial return, the investment in moxidectin would have been previously inconceivable, particularly as the registered treatment option for onchocerciasis (ivermectin) is donated. GHIF and MDGH, both with constitutionally enshrined global health objectives, have entered into a legally binding commitment to use PRV funds to support equitable and sustainable access to moxidectin for onchocerciasis, as well as support further development of moxidectin for other NTDs. In conclusion, this is the first example of a program utilizing the PRV scheme to support NTD drug development and access, directly addressing the majority of concerns raised about the use of PRV scheme to achieve global health impact.

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EVALUATING LONG-LASTING INSECTICIDAL NET EFFECTIVENESS OVER TIME USING SENTINEL SURVEILLANCE NETWORK: EVIDENCE FROM MADAGASCAR

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The reduction of global malaria burden over the past 15 years can be attributed to an unprecedented scale-up of malaria control interventions, particularly through mass distribution campaigns (MDCs) of long-lasting insecticidal nets (LLIN). Three LLIN MDCs were implemented in Madagascar at the end of 2009, 2012 and 2015 (coverage ranges from 80% to 94%). While malaria decline in Madagascar suggests the impact of MCIs, instances of malaria outbreaks between LLIN MDCs (in the absence of routine continuous distribution channels) suggest that diminished LLIN coverage may exist or net effectiveness may decline faster than expected. We conducted a study based on sentinel surveillance at 17 sites to assess the relationship between the effectiveness of LLIN MDCs over time and malaria outbreaks identified in Madagascar from 2009 to 2015. The association was evaluated using Generalized Linear regression Model

(GLM) and survival analysis. An alert was defined as weekly malaria cases exceeding the 90th percentile value for the three previous consecutive weeks. The percentile value is not seasonally-dependent and calculated over the whole chronological series of a site. GLM analysis showed that compared to the first year after a LLIN MDC, the probability of a malaria risk alert at surveillance sites increased dramatically during the second year (OR 37.9, 95% C.I 15.9-123.7) and further increased during the third year (OR 54.2, 95% C.I. 22.6-177.5). The survival analysis showed that each year that followed an LLIN MDC was alert free. Within two years after LLIN MDC, 51.4% (18/35) of sites were affected by an outbreak, and 77% (27/35) after 2 years. Data from Malagasy sentinel surveillance were valuable in assessing the effectiveness of LLIN mass campaigns. We provide evidence that LLIN MDC prevent malaria outbreaks although over time the frequency of outbreak alerts increased. Approaches for continuous LLIN distributions to maintain high coverage between MDCs are likely necessary to accelerate malaria control in Madagascar. Further studies are needed to investigate other causes responsible for the increase in malaria alerts over time after LLIN MDCs.

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A FRAMEWORK FOR THE SYSTEMATIC EVALUATION OF SEVERE DISEASE SURVEILLANCE SYSTEMS: BANGLADESH AS A CASE STUDY

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The International Health Regulations (IHR) outline the core requirements to ensure the timely detection of public health threats of international concern. Assessing the sensitivity and representativeness of surveillance systems to detect these threats is crucial to quantify the capacity to detect outbreaks and to interpret case statistics. We propose a framework for the systematic evaluation of severe disease surveillance systems and apply it to assess severe neurological and respiratory diseases surveillance in Bangladesh at tertiary care hospitals. During 2009- 2013, all cases of severe neurological and respiratory diseases were identified in surveillance hospital catchment areas outside of the capital city using key informant and house-to-house surveys. We ascertained where cases had sought care. We then estimated the probability of the surveillance system detecting case-clusters of varying size by distance from hospitals using a statistical algorithm and compared characteristics of cases identified in the community to the subset that sought care at surveillance hospitals. An estimated 25% of severe neurological and 16% of severe respiratory cases residing at 10km from the surveillance hospital sought care at those facilities. Outbreak detection probabilities decreased with distance from the hospital such that a cluster of severe respiratory disease occurring 30km from the hospital would be detected 90% of the time only if it included >30 cases. Characteristics of cases from surveillance were largely representative of all cases, however, <5 year-old children and lowest socioeconomic neurological cases and severe respiratory cases aged ≥60 were underrepresented (absolute difference 19%, 13%, 16%, respectively). Our study identified weaknesses of this system in detecting small-to-medium sized outbreaks at >30km distance from surveillance sites, likely because of limited access to healthcare in rural areas. These findings highlight difficulties that low and middle income countries may have in meeting IHR requirements, despite considerable investment in hospital-based surveillance platforms.

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KNOWLEDGE, ATTITUDES AND PRACTICES (KAP) ASSESSMENT OF MALARIA INTERVENTIONS IN ZAMBIA

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Despite the rapid upscale of malaria control interventions, such as long-lasting insecticidal nets and indoor residual spraying, malaria remains a major source of morbidity and mortality in Zambia. A comprehensive understanding of community knowledge, attitudes and practices (KAP) is a crucial component for enhancing the uptake and use of current and novel malaria control interventions for sustained disease prevention. The overall objective of this study was to assess malaria-related KAP, as well as entomological factors associated with the use and acceptability of malaria vector control interventions in a select cohort of caregivers (n=75) within Luangwa and Nyimba districts, eastern Zambia. Specific aims focused on assessing acceptance and/or use of currently deployed malaria interventions in relation to: 1) socio-demographic factors (education, age and occupation); 2) knowledge of malaria disease and function of available interventions; 3) cultural attitudes regarding perceptions of disease risk, the barriers to and benefits of intervention use; 4) cultural practices (outdoor sleeping and cooking); and 5) indoor densities of mosquitoes in study households. Similar data were generated and analyzed for assessing potential acceptance and use of a novel intervention currently being evaluated for malaria control, specifically a spatial repellent. Methodologies included the use of questionnaires and in-depth interviews as well as mosquito collections using CDC light traps. Findings from this study are anticipated to benefit the Zambian Ministry of Health's malaria education and vector control campaigns in these study sites.

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SITUATIONAL ANALYSIS OF MENTAL HEALTH ON EBOLA VIRUS DISEASE (EVD) AFFECTED COUNTRIES' VULNERABILITY USING THE PRESSURE AND RELEASE MODEL

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Prior to the 2013 West Africa Ebola outbreak, there were high rates of Post-Traumatic Stress Disorder (PTSD), intimate partner violence, and poor psychosocial adjustment in Liberia, Sierra Leone, and Guinea as an aftermath of two decades of civil war and political instability. Yet a low level of economic development contributed to poor access to health care and a high unmet need for treatment, particularly for PTSD. We adapted Blaikie's Pressure and Release (PAR) Model for disasters to apply it to a situational analysis of mental health factors that contributed to the initially poor Ebola Virus Disease (EVD) response in West Africa. The PAR Model assesses the contribution of political, economic, and environmental vulnerabilities as root causes for socio-economic pressures that create unsafe conditions. We posited that systemic vulnerabilities in the three countries contributed to the epidemic: A traumatized population evidenced distrust for existing institutions, yielding low levels of cooperation with public health authorities early in the outbreak, resulting in a delayed response to EVD. As it is highly likely that the EVD outbreak has increased trauma and economic losses in a population already burdened with high rates of mental illness, there has never been a more crucial time to address mental health needs in West Africa to reduce societal vulnerability and decrease maladaptive responses to hazards inherent in the environment. Community based services focusing

on building psycho-social and economic resilience can rebuild trust and lessen poverty, supporting healthy individual and societal adaption to change. The PAR model provides an effective tool for situational analysis by highlighting vulnerabilities and suggesting interventions to mitigate or prevent future health-related or natural disasters.

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HIGH PREVALENCE OF DUFFY-NEGATIVITY AMONG INDIVIDUALS INFECTED WITH *PLASMODIUM VIVAX* AND *P. OVALE* IN NORTHWEST ETHIOPIA

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The Duffy blood group antigen (Fy) is expressed on the surface of red blood cells and encoded by the Duffy Antigen Receptor for Chemokines (DARC) gene. Until very recently it was considered to be essential as a portal of entry of the *Plasmodium vivax* parasite into human red blood cells. Recent data suggest that this theory may no longer hold true and that in some parts of the world, *P. vivax* infects and causes disease in Duffy-negative individuals. Ethiopia reports one of the highest *P. vivax* malaria burdens in the world and the role the Duffy blood group is playing remains poorly understood. A total of 122 randomly selected samples from *P. vivax*-positive individuals originating from a region of high malaria endemicity and host genetic diversity in northwestern Ethiopia were successfully genotyped at the -33rd (promoter region SNP) and the 125th (A/B SNP) nucleotide positions. Another 47 samples from *P. vivax*-negative individuals from the same region as well as 15 samples from Asian individuals were tested as controls. Eleven out of 122 (9.0%) *P. vivax* malaria patients tested Duffy-negative (FY*BES/*BES). The allele frequencies were 18.8, 34.8, 0, and 46.3% for FY*A, FY*B, FY*AES, and FY*BES, respectively. The majority of the test samples (91/122; 74.6%) were heterozygous in the promoter region with the most common (58/122; 47.5%) genotype being FY*B/*BES. With almost 50% (23/47) the proportion of Duffy-negative individuals was significantly (OR=9.67; P<0.001) higher among *P. vivax*-negative controls from the same region. Interestingly among a small subgroup (N=10) of *P. ovale*-positive controls the proportion of Duffy-negative individuals was 70%. Although the FY*BES/*BES genotype seems to confer a certain level of protection against *P. vivax* malaria in Ethiopia, this protection seems to be far from universal. The fact that the population of northern Ethiopia is genetically highly heterogeneous may allow for the circulation of *P. vivax* parasites in host populations that otherwise may not be able to sustain long term transmission of *P. vivax*.

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EXPRESSION OF *PLASMODIUM FALCIPARUM* GENES INVOLVED IN ERYTHROCYTE INVASION, IMMUNE RESPONSES AND CLINICAL OUTCOMES

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Red blood cell (RBC) invasion is a key step in the *Plasmodium falciparum*'s life cycle and many vaccine candidates are proteins involved in this process. However, redundancy in protein function leads to different pathways used to invade and therefore, to a high diversity in ligand expression, highlighting the need of studying gene expression profiles in field isolates. We hypothesize that differences in gene expression are associated with immune responses to the proteins expressed or with the clinical outcomes of the infection (severe (SM) / uncomplicated (UM) malaria). Therefore, we sought to study the expression of genes involved in the invasion process in clinical isolates from infected individuals in Mozambique (adults with UM n=50; children with UM n= 25 or SM n=25). Transcript levels of *P. falciparum* genes *pfrh1, pfrh2a, pfrh2b, pfrh4, pfrh5, eba175, eba140, aarp, ptramp, p41, cyrpa* and *ama1* were determined using quantitative Polymerase-Chain Reaction (qPCR) and IgG/IgM levels against the resulting proteins were measured by Luminex. Preliminary results show a negative correlation between antibodies to some proteins participating in one invasion pathway (SA dependent or independent) and expression of genes involved in the other: IgG to EBA175 PfF2 and *pfrh2a* expression; IgG to $PfRh2_{2030}$ and *eba140* expression; IgG to $PfRh2_{40}$ and *eba140* expression; IgM to EBA175 III-V and *pfrh4* expression; IgM to EBA175 PfF2 and *pfrh4* expression. Also, we report a negative correlation between *ptramp, eba140, pfrh2b, pfrh4, pfrh5* and age, while *p41* expression is higher in older individuals. When accounted for "immune pressure" we found *aarp* expression at higher levels with higher immune pressure, whereas *pfrh2a* and *pfrh4* expression was lower. We also found that *p41* negatively correlated with *pfrh5* (rho -0,49; p<0,0001) and positively correlated with IgG against PfRh5. Data analysis is still ongoing. The greatest impact of this work will be on vaccine development: the effectivity of a vaccine designed to target RBC invasion could depend on the expression of candidate genes, which could be influenced by immunity against the ligands, as we suggest in this study.

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A LAP-LIKE PROCESS AS A NOVEL IMMUNE MECHANISM DOWNSTREAM OF IFN-γ IN THE CONTROL OF THE HUMAN MALARIA *PLASMODIUM VIVAX* LIVER STAGE

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Interferon-gamma (IFN-γ) is a main regulator of immune functions and has been previously shown to induce *Plasmodium* liver stage elimination both in *in vitro* and *in vivo*. However, the molecular mechanism responsible for the restriction of *Plasmodium* liver stage downstream of IFN-γ remains uncertain. Recently autophagy, a newly described immune defense mechanism, was identified as a downstream pathway in response to IFN-γ in the control of intracellular infections. We, therefore, hypothesized that the killing of liver stage malaria parasites by IFN-γ may be through autophagy induction. Our results showed that while IFN-γ treatment of human hepatocytes activates autophagy, IFN-γ-mediated *P. vivax* liver stage restriction only requires downstream autophagy-related proteins Beclin 1, PI3K, and ATG5 but not the upstream autophagy-initiating protein ULK1. In addition, an enhanced recruitment of LC3 onto the parasitophorous vacuole membrane (PVM) and an increased colocalization of lysosomal vesicles with *P. vivax* compartments were observed in response to IFN-γ. Altogether, these data indicated that IFN-γ mediates the control of *P. vivax* liver stage by inducing a noncanonical autophagy pathway resembling that of LAP in which LC3 is directly decorated onto the PVM and mediates the fusion of *P. vivax* compartments with lysosomes resulting in the killing of the pathogens. Understanding hepatocyte response to IFN-γ during *Plasmodium* infection and the roles of autophagy-related proteins may provide an alternative strategy urgently needed for the elimination of this human malaria.

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ASSOCIATION OF HOST GENETIC POLYMORPHISMS WITH PROTECTION AGAINST SEVERE MALARIA IN UGANDAN CHILDREN

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Human genetic polymorphisms are associated with risk of severe malaria. We compared the prevalence of selected host polymorphisms in children aged 4 months to 10 years enrolled in a case-control study in Jinja District, Uganda. Healthy controls (HC) and children with uncomplicated malaria (UM) were matched by date and location with children with severe malaria (SM) diagnosed based on standard WHO criteria; 305 children were enrolled in each group. Malaria was diagnosed based on fever and a positive Giemsa-stained smear. Genes of interest were amplified from purified DNA, polymorphisms identified by standard methods, and prevalences compared by Fisher's exact test. We present preliminary results for the first 100 cases and controls. For two studied alleles, the prevalence of WT was significantly greater in children with SM compared to UM (α-thalassemia 3.7kb deletion 60% in SM vs. 42% in UM, p = 0.0193; CD36 T188G 84% in SM vs. 38% in UM, p<0.0001) or compared toHC (α-thalassemia 60% in SM vs. 43% in HC, p = 0.0290; CD36 T188G 84% in SM vs. 55% in UM, p<0.0001). Non-significant trends toward a greater prevalence of WT with SM were also seen for sickle hemoglobin (β-globin E6V 92% in SM vs. 86% in UM, p=0.25 and vs. 87% in HC, p=0.36) and ICAM1K29M (61% in SM vs. 55% in UM, p=0.46 and vs. 48% in HC, p=0.063), but not the G6PD A- genotype (80% in SM vs. 79% in UM, p=1.0 and vs. 83% in HC, p=0.58) or CD36 T1264G (85% in SM vs. 82% in UM, p=0.70 and vs. 82% in HC, p=0.70). Our results suggest associationsin Ugandan childrenbetween some previously studied polymorphisms and protection against severe malaria, and in particular suggest a marked protective effect of the CD36 T188G genotype. Analysis of our full study population and consideration of different clinical presentations of SM are underway.

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THE IMPACT OF PRENATAL MALARIA EXPOSURE ON THE VULNERABILITY OF OFFSPRING TO NEUROPSYCHIATRIC DISORDERS

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Each year ~125 million pregnant women are at risk for malaria infection. Malaria in pregnancy (MiP) has a profound impact on mother-child health, including delivery of low birth weight (LBW) infants. Even in the absence of LBW, epidemiological studies have shown a connection between maternal infections during pregnancy and increased susceptibility of offspring to neuropsychiatric disorders later in life. Moreover, immune activation in pregnancy has been shown to prime the offspring to psychological trauma, showing a synergistic effect with stress-induced neuropsychiatric disorders. Mental illness represents the leading cause of DALYs globally and exerts a huge burden in malaria-endemic areas, but the impact of malaria exposure *in utero* on long-term vulnerability to psychiatric diseases has not been reported. We hypothesize that prenatal exposure to MiP will increase the risk of neuropsychiatric disorders in offspring. We used the established mouse model of MiP with *Plasmodium berghei* ANKA (PbA). Uninfected offspring of dams infected with an inoculum of PbA that does not induce a birth phenotype, were subjected to stressors or control handling at peripubertal age. Adult behavioural outcomes were assessed using standardized neuropsychiatric tests, including prepulse inhibition, open field, and amphetamine hypersensitivity. MRI, neurochemistry and levels of neuroinflammation were monitored to define correlates of susceptibility. Recent work by our lab showed that interrupting pathways of complement activation (i.e. C5a) and dysregulated angiogenesis (i.e. decreased Ang-1) improved fetal outcome in the MiP mouse model. We will therefore compare the neuropsychiatric outcomes in α-C5a/rAng-1 treated and untreated offspring as putative interventions. This concept represents a paradigm shift in our current understanding of the risk factors for neuropsychiatric disorders and identifies PM as a potential modifiable risk factor. This has important implications for the cause and prevention of what are believed to be "non-communicable" diseases, and may shift global health priorities from costly rehabilitation to prevention.

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EARLY AND LATE *PLASMODIUM FALCIPARUM* GAMETOCYTES-SPECIFIC EXPRESSION FOLLOWING TREATMENT WITH CHLOROQUINE AND SULPHADOXINE/ PYREMITHAMINE

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Elevation of sexual stages of *Plasmodium falciparum* following anti-malarial treatment has been attributed to either induction of gametocytogenesis, or release of sequestrated gametocytes in deep tissues. Attempts to address this question have been impaired by the unavailability of markers to specifically detect and quantify expression of early stages gametocyte (I-III) in peripheral blood. We have established and validated RT-qPCR assays to detect and quantify genes specifically expressed in early gametocytes and used them alongside a known mature gametocyte-specific marker (*pfs25*) to monitor gametocytogensis following treatment of *P. falciparum* infections with CQ or SP. The density of early and mature gametocytes and its ratio to total parasite density pre-treatment (D0) were compared to that seen post-treatment on D7, D14 and D21 among patients treated with different drug regimens (CQ or SP) and among different parasitological responses (sensitive and resistant [RI and RIII]). Prevalence and density of early gametocytes as well as early gametocyte parasite ratio (EGPR) decreased following treatment (D7), paralleled to parasite density, among all treatment groups and parasitological responses. However, EGPR increased significantly on D14 irrespective of the treatment regimen or parasitological response, which suggests an enhanced gametocytogenesis. Density but not prevalence of mature gametocytes increased on D7 and D14 post-treatment, regardless of drug treatment regimen or parasitological response, suggesting release of sequestrated gametocytes that were not affected by treatment on D0. This study demonstrated evidences for increasing commitment to gametocytogenesis following anti-malarial therapy as well as possible release of sequestrated gametocytes.

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COMBATING ANEMIA WITH IRON SUPPLEMENTATION MAY INEVITABLY CAUSE A TRANSIENT INCREASE IN MALARIA RISK

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Epidemiological studies suggest iron deficiency protects against malaria and administering iron to iron-deficient individuals may increase malaria risk. This has generated much debate in the public health field about how to best distribute iron supplements to anemic populations in malaria endemic areas. Our previous laboratory work demonstrated decreased *Plasmodium falciparum* growth in iron deficient red blood cells (RBCs) and increased infection susceptibility in young RBCs and reticulocytes *in vitro*. Here, our objective was to comprehensively evaluate *P. falciparum*

pathogenesis in iron deficient children and pregnant women before, during, and after iron supplementation. We also sought to evaluate the hypothesis that malaria risk increases as erythropoiesis increases in response to iron supplementation. To do so, we investigated *P. falciparum in vitro* growth characteristics in RBCs from Gambians participating in iron supplementation trials. RBCs were collected from 135 children (ages 6-24 months; hemoglobin levels < 11 g/dL) and 165 pregnant women (2nd and 3rd trimester) before, during, and upon completion of 12 weeks of iron supplementation (12 mg or 60 mg daily, respectively). Using flow cytometry-based assays, we separately examined effects of iron deficiency and iron supplementation on overall parasite growth and merozoite RBC invasion. Our results demonstrate *P. falciparum* erythrocytic stage growth *in vitro* is low at baseline and increases during supplementation using RBCs from both the children and pregnant women. Additionally, we show parasite invasion is reduced in iron deficient RBCs from Gambian children and increases during iron supplementation. The elevated growth rates parallel increases in circulating reticulocytes and RBC mean corpuscular hemoglobin concentration, the kinetics of which correlate with increased erythropoiesis. We conclude malaria growth *in vitro* corresponds with elevated erythropoiesis, an inevitable consequence of iron supplementation. Our findings imply iron supplementation in malarious regions should be accompanied by effective preventative measures against falciparum malaria.

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CD155, THE POLIOVIRUS RECEPTOR, NEGATIVELY REGULATES IMMUNE PROTECTION TO *PLASMODIUM YOELII* 17XNL MURINE MALARIA

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CD155, the cell entry receptor for poliovirus, is a member of a large family of immunoglobulin-like molecules called nectins and nectin-like proteins. Recently, CD155 has been ascribed several important immunological functions. For example, CD155 expression on target cells is essential for natural killer and $CD8^+$ T cell mediated lysis of certain tumor cells. In addition, CD155 promotes Th1 development from naïve $CD4^+$ T cells and can significantly alter the humoral immune response to invading pathogens. We measured the effect of CD155 on the immune response and pathogenesis following infection with a highly virulent and avirulent malaria in mice deficient for the *CD155* gene. In the *Plasmodium berghei* ANKA murine model of experimental cerebral malaria (ECM), there was no significant difference in susceptibility to ECM or parasitemia between CD155-deficient mice and wild type (WT) controls. In contrast, loss of *CD155* resulted in a 2.8 fold decrease in peak parasite burden on day 10 post-infection in the *P. yoelii* 17XNL, self-resolving and non-lethal malaria, suggesting that CD155 hinders the immune clearance of *P. yoelii* 17XNL. Since CD155 can serve as a ligand for the CD96, CD226, and TIGIT receptors, functional studies are currently underway to determine which interaction is responsible for CD155 mediated regulation of *P. yoelii* 17XNL parasite burden. In addition, key differences in immune cell subsets measured by in depth flow cytometric analysis and the cytokine and antibody profiles determined by ELISA between WT and CD155 knockout mice over the course of a *P. yoelii* 17XNL infection will be presented.

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PLASMODIUM FALCIPARUM PARASITES THAT INFECT RETINOPATHY POSITIVE AND NEGATIVE CEREBRAL MALARIA CHILDREN HAVE SIMILAR TRANSCRIPT ABUNDANCE OF VAR GENES ASSOCIATED WITH SEVERE MALARIA

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An accurate diagnosis of cerebral malaria (CM) is important for understanding the pathogenesis of CM. Malarial retinopathy, which is seen in some (retinopathy-positive, RP) children but not others (retinopathy-negative, RN) with clinical CM (coma with *P. falciparum* on blood smear) has been associated with parasite sequestration in the brain and is considered a strong indicator of "true" CM. However, it is unclear whether RN CM is a severe non-malarial illness with incidental parasitemia or a less severe form of RP CM. P.falciparum erythrocyte membrane protein-1 (PfEMP-1) is an important virulence factor in severe malaria and expression of group A var genes and more specifically domain cassette (DC) 8 and DC13 PfEMP-1 have been associated with severe malaria. Characterizing var gene expression in both RN and RP CM children could help elucidate the role of P.falciparum in RN CM pathogenesis. In the present study we performed RT-quantitative PCR using degenerate primers amplifying var genes encoding for 13 different PfEMP-1 domains. Testing was performed on RNA isolated from whole blood of Ugandan children with RP CM (N=39), RN CM (N=35), severe malarial anemia (SMA, N=39) and asymptomatic children (AC, N=12). Transcript abundance of var genes encoding for DC8, DC13 and other group A domains (CIDRα1.7) was higher in children with severe malaria than in asymptomatically infected community children ($P<0.007$ for all). The prevalence of DC8 and DC13 high transcribers was similar between RP and RN CM children (74.4% vs. 68.5 %, P=0.58 for DC8 and 53.8% vs. 37.1%, P=0.15 for DC13). RN children had lower median transcript abundance for DC13 (median arbitrary units Tu 4.16, interquartile range (IQR) 1-11.12) than children with RP CM (median Tu 8.08, IQR 2.43-20.31, P=0.04) but similar to SMA (median Tu 1.11, IQR 1-10.51, P=0.43) and higher than asymptomatically infected children (median Tu 1, IQR 1-1, P=0.0009). In our cohort, the parasites infecting RN CM children have similar transcript abundance for var subtypes associated with binding and severe disease as compared to RP CM, suggesting an important role for P.falciparum binding in the pathogenesis of RN CM.

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CONTINUOUS DETERMINATION OF BLOOD GLUCOSE IN ADMITTED CHILDREN WITH MALARIA IN A RURAL MOZAMBICAN HOSPITAL

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Hypoglycaemia is a frequent complication among admitted children, particularly in malaria-endemic areas. We aimed to estimate the incidence of hypoglycaemia throughout the hospitalization in children with malaria. A simple pilot study to monitor continuously glycaemia in children aged 0 to 10 years, admitted with malaria in a rural hospital was carried out in Southern Mozambique. Continuous glucose monitors (CGMs) were inserted in subcutaneous tissue of the abdominal area, producing glycaemia readings every 5 minutes. Glucose was continuously monitored during a mean of 48 hours, in 74 children. All of them were admitted

with malaria (22 severe, 52 uncomplicated). Five children (6.8%) had hypoglycemia (<3.0mmol/l) on admission as detected by routine capillary determination. Analyzing the data collected by the CGMs, we detected hypoglycemia episodes (<3.0mmol/l) in 11/74 (14.9%) children, of which, 8 (10.8%) could be classified as severe (≤2.5mmol)%). No differences in age, sex, malnutrition status and duration of hospital stay were found among hypoglycemic and normoglycemic children. Anemia was more common in hypoglycemic children. Only one death happened (1/74, 1.4%) among a normoglycaemic child. Hypoglycaemia beyond admission in children with malaria appears to be much more frequent than what had been previously described. The clinical relevance of these episodes of hypoglycemia in medium or long term remains unclear.

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ASSESSING THE PROTECTIVE ROLE OF NEUREGULIN 1 (NRG-1) AGAINST HEME-INDUCED TROPHOBLAST APOPTOSIS AND FUSION DAMAGE

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Syncytial trophoblasts (ST) Fusion and conversion from the mononucleated to the syncytial state are mandatory for successful pregnancy. In placental malaria, *Plasmodium falciparum* infected erythrocytes (IE) bind to trophoblasts resulting in inflammation and pathology associated with poor pregnancy outcomes. The interaction between ST and IE is reminiscent of the interaction that occurs in the brain leading to cerebral malaria. However, the role of free heme in the maintenance of the integrity of the placental barrier and the effects of free heme on pregnancy outcome are unclear. Recent studies have demonstrated the cyto-protective role of Neuregulin 1 (NRG-1) in brain vascular endothelial and neuroglia cells during cerebral malaria pathogenesis. We hypothesized that apoptosis and fusion of trophoblast-derived choriocarcinoma cell line (BeWo) will be attenuated by NRG-1In the present study we determined the effect and molecular mechanisms of heme on the syncytial fusion triggered by forskolin using the BeWo cell line. Results of this study demonstrated that 1). Heme induces BeWo cells apoptosis which is attenuated in the presence of NRG-1 2). Heme induces apoptosis of BeWo cells through activation of STAT3/caspase-3/PARP and P73 signaling pathways. 3). BeWo cell fusion is reduced in the presence of heme, 4).Heme reduces mRNA expressions of cell-fusion related genes when forskolin induces syncytialisation, 5). Heme inhibits differentiation and fusion of BeWo cells through activation of STAT3 signaling pathways. 6)NRG-1 against heme-induced trophoblast apoptosis and fusion damage. In conclusion, we identified a nove mechanism responsible for the inhibition of trophoblast fusion by heme. In the absence of forskolin, heme induced apoptosis in BeWo cells via activation of STST3/caspase-3/APARP; while in the presence of forskolin, pSTAT3 was induced by heme to inhibit cell fusion. Our studies indicated that heme-induced pathways may be attenuated by NRG-1 and therefore could be potential drug targets in the prevention of heme-associted trophoblast cell apoptosis and fusion damage.

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PREVALENCE OF PFCRT, MOLECULAR MARKER OF RESISTANCE OF *PLASMODIUM FALCIPARUM* ISOLATED FROM PATIENTS WITH UNCOMPLICATED MALARIA IN DAKAR

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The malaria remains the major parasitic disease affecting for Human. Nearly 40 % of the world population lives in an area at risk of infection. In Senegal a new strategy called Seasonal Malaria Chemoprevention (SMC) is currently implemented in four districts in the center and the south of the country. However, the protective efficacy of these strategies depends heavily on the current level of resistance to Sulfadoxine-Pyrimethamine (SP) and its spread among populations of *Plasmodium falciparum*. Resistance of *P. falciparum* to this molecule is well established in East Africa and continues to spread westward. It is shown further that Amodiaquine (AQ) associated with SP in the SMC had the same action as Chloroquine (CQ) and malaria strains resistant to CQ became resistant to AQ. It is in this context that our work objective was to provide information on the impact of control strategies against malaria on molecular markers of resistance, this for a good orientation of policy making malaria burden and thereby strengthen malaria control programs. The specific objective was to re-evaluate the prevalence of mutations associated with resistance of *P. falciparum* to CQ after the abandonment of this molecule in the treatment of uncomplicated malaria in Senegal. Blood samples on filter paper were made in patients with uncomplicated malaria and treated with ACTs as ASAQ, Duo-Cotexcin and Coartem in two health districts in Dakar. We have identified mutations in the pfcrt gene by PCR- RFLP. Result In this study, men were more represented than women, 63% and 37% respectively. Pfcrt mutation gene is more pronounced among women within all treatment groups. Also our study showed return susceptible strains to Chloroquine (75% of wild strains), molecular analysis showed that among the 160 patients analyzed 25 % of patients had a mutation located on the pfcrt gene. In conclusion, this study showed a relatively high prevalence compared to WHO standards which provides for a maximum transfer of 10%. This is a sign of resistance to antimalarial *P. falciparum* face hence the need to implement the ACT resistance surveillance studies.

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A NOVEL CLASS OF METABOLIC REGULATORS MEDIATE FOSMIDOMYCIN SENSITIVITY AND RESISTANCE IN MALARIA PARASITES

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Drug resistance remains a significant challenge in malaria control. Isoprenoid synthesis via the methylerythritol phosphate (MEP) pathway is essential for parasite survival and is a proven antimalarial drug target. The phosphonic acid antibiotic fosmidomycin (FSM) is a well-characterized inhibitor of this pathway and is currently in Phase II clinical trials. In a forward selection for FSM resistance, we identify a loss-of-function mutation in PfHAD2, a member of the haloacid dehalogenase-like hydrolase (HAD) superfamily, as the resistance-causing mutation. Enzymatic characterization of PfHAD2 shows that it is a purine monophosphatase. Using metabolic profiling, we show that loss of PfHAD2 results in increased levels of MEP pathway metabolites, allowing the parasites to overcome pathway inhibition by FSM. PfHAD2 mutants also have a growth defect, allowing for selection of suppressors of PfHAD2-mediated resistance. We identify hypomorphic mutations in the glycolytic enzyme phosphofructokinase (PfPFK9) that suppress HAD2-mediated resistance. This points to PfHAD2 as a novel regulator of essential central carbon metabolism, and dysregulation of this metabolism mediates drug sensitivity. Our work represents a novel use of forward genetics in *Plasmodium falciparum* to better understand drug resistance, metabolism, and the function of this previously undescribed protein class. PfHAD2, PfPFK9, and other regulators of essential metabolism may function as future targets for antimalarials.

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PREVALENCE OF MOLECULAR MARKERS OF *PLASMODIUM FALCIPARUM* RESISTANCE TO SULPHADOXINE/ PYRIMETHAMINE IN CHILDREN WITH SICKLE CELL ANAEMIA AGED 6 TO 59 MONTHS IN BENIN CITY

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Sulphadoxine/Pyrimethamine (SP) for Seasonal Malaria Chemoprevention (SMC) or Intermittent Preventive Therapy (IPT) has been shown to be efficacious and effective in preventing malarial induced morbidities and deaths in infants and young children including those with sickle cell anaemia (SCA). However, mutations in *Plasmodium falciparum dihydrofolate reductase (pfdhfr) and dihydropteroate synthase (pfdhps)* genes, resulting in SP resistance pose a threat to its efficacy. Little is known about the prevalence of these mutations in malaria parasites infecting children with SCA, who are usually on proguanil as chemoprophylaxis (which has been found to be ineffective). To determine the pattern and prevalence of *pfdhps* and *pfdhfr* mutations in children with SCA infected with *P.falciparum*, we enrolled 146 (71 children with SCA and 75 children without SCA) children. Genomic DNA was extracted from one hundred and forty six filter paper bloodspots and point mutations at codons 431, 436, 437, 540, 581 and 613 of the *pfdhps* gene and codons 16, 51, 59, 108, and 164 of the *pfdhfr* gene were evaluated by nested Polymerase chain reaction amplification followed by direct sequencing. In children with SCA, the prevalence of *pfdhps* S436A, A581G and A613S mutations were 40(56.0%), 31(44.0%), and 30(42.0%) respectively; while children without SCA had a statistically significantly higher prevalence: 54(72.0%), 52(69.3%), and 53(70.7%) respectively (p=0.048 for A436S, p=0.002 for A581G, p=0.001 for A613S). The *pfdhps* K540E mutation and *pfdhps* double mutant haplotype (A437G+K540E), which confer significant resistance to SP were not found in this study. The prevalence of the emerging *pfdhps* mutant haplotype VAGKGS was significantly higher in the non-SCA group (p<0.001). The prevalence of *pfdhfr* triple mutant haplotype: N51I+C59R+108N was similar in both study groups. The deployment of SP for malaria chemoprevention in infants and young children with SCA can be explored in Nigeria as an alternative to proguanil. Parasites with mutations on the *pfdhps* gene are more likely to be found in children without SCA than in those with SCA.

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SELECTIVE SWEEPS AND GENETIC LINEAGES OF *PLASMODIUM FALCIPARUM* MULTI-DRUG RESISTANCE (PFMDR1) GENE IN KENYA

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Artemether-lumefantrine (AL) has been the first-line treatment for uncomplicated falciparum malaria in Kenya since 2006. However, there are concerns that resistance to current drugs might emerge as has been reported along the Thailand-Cambodia border. Single nucleotide polymorphisms (SNPs) in critical alleles of Pfmdr1 gene has been associated with resistance to Artemisinin and its partner drugs. AL selects for K76 in Pfcrt and N86, 184F and D1246 in Pfmdr1 genes in recurring parasites compared to the baseline infections. Microsatellite analysis of loci flanking genes associated with antimalarial drug resistance has been used in defining the geographic origins, dissemination of resistant parasites and identifying regions in the genome that have been under selection. This study investigated evidence of selection in Pfmdr1 genotypes selected for by AL in treatment of malaria infections in Kenya and their genetic lineages. Parasites (n=252) from different regions in Kenya were assayed for SNPs at codons 86, 184 and 1246 and typed for 7 neutral microsatellites (NMS) and 13 microsatellites loci flanking (±99 kb) Pfmdr1 in *Plasmodium falciparum* infections. Full data sets of pure SNP genotypic data were obtained in 132 of the samples. Overall, the prevalence of N86 and D1246 was highest at 86.4% and 93.9% respectively. Single mutant NFD was the most prevalent haplotype at 44.7%, whereas the least prevalent were double mutants YFD and NFY both at 0.8%. The mean Expected heterozygosity (He) for NMS was 0.908 vs. 0.535 for the 8 closest MS indicating selection. Parasites carrying mutant alleles had reduced He compared to the NMS and wild type. Analysis of parasite genetic lineages showed that mutant parasites genotypes came from multiple genetic backgrounds. Data show a generally high prevalence of NFD and NYD, difference in genetic diversity between sites and little reduction of He in NYD and NFD from NMS. The reduction of He in YYD is however significant (P=0.0001). Data indicates parasites are evolving differently in response to AL drug pressure in the different regions suggesting the rate at which AL tolerance will develop in different regions of Kenya might vary.

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IN VITRO CHEMO-SENSITIVITY OF *PLASMODIUM FALCIPARUM* ISOLATES ON DAY 0 PRIOR TO TREATMENT DURING PHASE IIIB/IV CLINICAL STUDY IN BOBO DIOULASSO (BURKINA FASO)

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Artemisinin-based combination therapy (ACT) is used to treat all cases of uncomplicated malaria in Burkina Faso. In this study, 50% inhibitory concentration to determine the cut-off values for *in vitro* reduced susceptibility of Chloroquine (CQ), Dihydroartemisinin (DHA), Pyronaridine (PYD) and Piperaquine (PIP) for *Plasmodium falciparum* isolates from a phase IIIb/IV comparative 3-arm clinical study to assess the safety and efficacy of repeated administration of pyronaridine-artesunate, dihydroartemisinin-piperaquine or artemether-lumefantrine or artesunate-amodiaquine over a two-year period in children and adult patients with acute uncomplicated *Plasmodium* sp. malaria. *In vitro* susceptibility on day 0 prior treatments was assessed by the standard WWARN protocol. Each isolate was tested in triplicate by using pre-coated microplates with serial dilutions of CQ, DHA, PYD and PIP. The results were analysed on HNnonlin software and expressed as the 50% inhibitory concentration (IC50) or geometric mean IC50 (GMIC50). CQ IC50 values ranged from 25 to 464.89 nM, with a geometric mean of 63.34 nM. DHA IC50 values ranged from 0.33 to 12 nM, with a geometric mean of 1.16 nM. PYD IC50 values ranged from 1,250 to 25, 35 nM, with a geometric mean of 57.21 nM. PIP IC50 values ranged from 5 to 230.5 nM, with a geometric mean of 13.16 nM. In conclusion, around 70-90 % isolates were sensitive to CQ and DHA, but only 57% for PIP. Pyronaridine showed susceptibility of 14% on isolates tested. The risk of emerging resistance to ACT in West Africa makes necessary to continue monitor the susceptibilities of parasites to antimalarial drugs, particularly those used in combination with artemisinin derivatives.

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ROLE OF ANTIMALARIAL DRUG CONCENTRATION IN DE NOVO SELECTION OF DRUG TOLERANT *PLASMODIUM FALCIPARUM* PARASITE STRAINS *IN VITRO*

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Malaria is one of the most devastating infectious diseases, with the emergence of antimalarial drug resistance being of great concern. Availability of effective antimalarial drugs is quickly dwindling due to

different causes. Several factors have been attributed to drug resistance. However inadequate antimalarial drug concentration in blood plasma has been identified as a major cause. When exposed to a drug, sensitive parasites die while tolerant parasites survive leading to subsequent transmission. Genetic identity of these strains could give an answer to occurrences of treatment failure.This study seeks to establish how different antimalarial concentration can contribute in selection of drug tolerant parasite strains *in vitro*. It will utilize *Plasmodium falciparum* samples from an ongoing ethically approved clinical trial in holoendemic sites. Each admission sample (day 0) will be exposed to 70 nanomolar (nM) and 700nM dihydroartemisinin (DHA) for 6hrs and compared with the parasites in drug-free (DMSO) wells. The 700nM DHA is the optimum clinical relevant concentration while 70nM DHA is 10 fold lower sufficient to produce a change in malaria treatment. The DHA-exposed sample will be washed 3 times and re-cultured for 66 hours. Aliquot of 200 µL will be obtained from each of 28 samples recruited at 24 and 72 hour in culture respectively. Extraction of genomic deoxyribonucleic acid (gDNA) will be done using Qiagen kit. To determine selection after 24hrs and 72 hrs in culture respectively, fragment analysis will be run on 12 loci using capillary electrophoresis. Single nucleotide polymorphisms (SNPs) of Pfcrt and Pfmdr1 and will be determined using MassArray platform and Sanger sequencing. Categorical data for respective SNPs will be analyzed using Kruskal-Wallis test. Heterozygosity (He) and genetic differentiation at the 2 time points and concentration will obtain using GenAlex software. He values obtained will be used to determine selection. The findings from this study may reveal useful genetic information associated with tolerant *Plasmodium* strains that likely to become precursors for drug resistance.

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PHYSIOLOGICALLY RELEVANT EXPOSURE TO SYNTHETIC OZONIDE ANTIMALARIALS KILLS K13 WILDTYPE AND MUTANT *PLASMODIUM FALCIPARUM* MORE EFFECTIVELY THAN DIHYDROARTEMISININ

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Fully synthetic endoperoxide antimalarials, namely OZ277 (RBx11160, arterolane) and OZ439 (artefenomel), have been approved for marketing or are currently in clinical development. We undertook an analysis of the kinetics of the *in vitro* responses of *Plasmodium falciparum* to the new ozonide antimalarials, using a K13 mutant (artemisinin resistant) isolate from a region in Cambodia and a genetically matched (artemisinin sensitive) K13 revertant. We used a pulsed exposure assay format to interrogate the time-dependence of the response. Because the ozonides have different physicochemical properties to the artemisinins, assay optimization was required to ensure that the drugs are completely removed following the pulsed exposure. Like the artemisinins, ozonide activity requires active hemoglobin degradation. Short pulses of the ozonides were less effective that dihydroartemisinin; however when early ring stage parasites are exposed to drugs for time periods relevant to their *in vivo* exposure, the ozonide antimalarials are markedly more effective.

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HISTORICAL AND CURRENT PATTERN OF ANTIMALARIAL DRUGS USE IN THE EPIDEMIC PRONE AREAS OF WESTERN KENYA

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Management of malaria by artemisinin based combination therapy (ACTs) averts the disease sequel and limits infection propagation. This study aimed at examining the historical and current clinical prescription pattern, household use and availability of antimalarial drugs in Western Kenya. Multiple community and hospital based cross-sectional studies in three sites were done on types of antimalarial used and those prescribed respectively during ACTs drug policy implementation. Random selection of participants and review outpatient hospital registers together with the assessment of antimalarial drug availability in registered drug outlets were done. The hospital ACT prescription compliance was effectively done (100% (4042/4042) by 2015 but the adoption of the new drug policy had some rise and fall. Back in 2007 the prescription was 60% (6,363/10306) compliant before it dropped to 54.2% (712/1313) in 2010. The monotherapy antimalarial drugs use in households had been subsequently decreasing to 5.7% (86/1500) in 2015. Majority of users (90.5% (76/84) were from epidemic prone areas [$\chi 2=27.54$; df =2; $p<0.001$] and most were above 14 years of age (70/84 (83.3%) [$\chi 2= 56.08$; df=2; $p<0.001$]. In 2015 survey, majority (76.7% (227/296) of the under five years obtained their antimalarial drugs from Government hospitals while nearly half (42.5% (281/661) of the above 14 years of age obtained theirs from community drug outlets [$\chi 2= 126.13$; df =4; $p<0.001$]. The surveyed community drug outlets in 2015 found with high availability of both ACTs (100% (59/59) and 81.4% (48/59) of sulphadoxine-pyremethamine monotherapy. Compliance to ACTs drug prescription in Kenyan Government health facilities had been staggering along the course but currently effective. Similarly, the trend of household ACTs use had been improving with some rise and falls but the observed magnitude of monotherapies use might be even higher as their availability in community drug outlets still high. Continued monitoring of AMD use both in health facilities as well as in the community along with reinforcement of the endorsed efficacious drug use is highly recommended.

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PREVALENCE OF CHLOROQUINE AND ANTIFOLATE DRUG RESISTANCE MUTATIONS IN *PLASMODIUM FALCIPARUM* FIELD ISOLATES FROM TWO AREAS IN GHANA

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As a result of widespread drug resistance to chloroquine and the antifolates among *Plasmodium falciparum* parasites, artesunate combination therapies (ACTs) were introduced as first line drugs in Ghana in 2005. However, the establishment of ACT resistance in South East Asia is an ominous sign, since this region is notorious for the emergence of antimalarial drug resistance, with possible spread to the African region. To investigate the prevalence of chloroquine and antifolate drug resistant

parasites in Ghana, we tested for *P. falciparum* drug resistance gene mutations using Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP). *P. falciparum* isolates were collected from 203 children aged 2-14 years from two areas in Ghana with different transmission patterns, Navrongo and Kintampo, during the peak seasons in 2012-2013. The proportions of isolates with the *pfcrt* T76 mutation were 13.7% and 11.9% in Kintampo and Navrongo respectively. The overall prevalence rate of T76 mutation was 8.9% in the 2012-2013 period, which is significantly reduced when compared to data from the World Wide Antimalarial Resistance Network (WWARN) showing 76-100% rate from 2000-2005. For *pfmdr1,* the Y86 polymorphism was 10.4% and 17.5% in Kintampo and Navrongo respectively, with the overall prevalence in the combined population being 12.8% as against 51-75% in 2007-2008 as captured in the WWARN database. However, drug resistance mediating gene polymorphisms in *pfdhfr* (N108, 88.4%, I51, 80.6%; R59, 82.5%) *and pfdhps* (G437, 88.2%, E540, 1.2%) were comparable to data from 2000-2005 on *pfdhfr* (N108, 51-75%, I51, 51-75%; R59, 76-100%) and *pfdhps* (G437, 76-100%; E540, 1-25%) polymorphisms. The reduction in the *pfcrt* T76 genotype in the Ghanaian population is attributable to the re-expansion of the wildtype genotype K76 as result of the proscription of chloroquine as a first line antimalarial drug in 2004. However, the use of antifolates for intermittent preventive treatment of malaria in pregnancy and for seasonal malaria chemotherapy in infancy may explain the persistence of antifolate drug resistant gene polymorphisms at higher frequencies.

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CHANGES IN DRUG RESISTANCE-MEDIATING *PLASMODIUM FALCIPARUM* POLYMORPHISMS IN UGANDA OVER TIME

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Antimalarial drug resistance, mediated in part by known *Plasmodium falciparum* genetic polymorphisms, is of great concern. Chloroquine plus sulfadoxine-pyrimethamine (SP) was replaced by artemether/lumefantrine (AL) as the national treatment regimen, beginning in 2006. SP remains the standard-of-care to prevent malaria in pregnant women. We hypothesized that altered selective pressure for drug resistance would lead to changes in resistance polymorphisms in Ugandan parasites. We used ligase detection reaction-fluorescent microsphere assays to analyze 1486 samples from cross-sectional surveys of subjects in 2012, 2013, and 2015 in Tororo, Jinja, and Kanungu districts for polymorphisms in the putative drug transporters *pfcrt* and *pfmdr1* that impact on response to AL and other drugs, and polymorphisms in the folate genes *pfdhfr* and *pfdhps* associated with resistance to SP. The prevalence of pure WT sequences was markedly greater than seen in Uganda previously, and increased from 2012 to 2015 for *pfcrt* K76T (3.0% to 28.6%), *pfmdr1* N86Y (33.9% to 84.5%), and *pfmdr1* D1246Y (45.6% to 74.4%); combined WT/mixed genotypes increased in a similar manner. For antifolates, the prevalence of 5 mutations (*pfdhfr* N51I, C59R, S108N; *pfdhps* A437G, K540E) that have been common since initial studies over a decade ago remained high (generally >90% mixed/mutant). Of concern, two additional mutations that predict a greater level of resistance to SP appear to be emerging in Uganda, with the prevalence of mixed/mutant *pfdhfr* I164L increasing from 7.3% to 11.3% and of *pfdhps* A581G from 29% to 35% in Kanungu, and mixed/mutant *pfdhps* A581G also seen commonly in Jinja (21.0%) and Tororo (14.1%) in 2015. Our results demonstrate significant changes in the prevalence of transporter polymorphisms with increasing use of AL to treat malaria, persistent prevalence of 5 common antifolate mutations despite decreased use of SP to treat malaria, and the presence of additional antifolate mutations that predict high level resistance. Continued surveillance for drug resistance markers is an important priority.

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MUTATION ANALYSIS OF K13 GENE, PFMDR1 AND PFCRT ∫G V IN *PLASMODIUM FALCIPARUM* ISOLATES COLLECTED FROM WESTERN KENYA

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In Southeast Asia (SEA), mutations in K13 gene have been shown to be artemisinin resistance key determinant. Single Nucleotide Polymorphisms (SNPs) within the K13-propeller domain confer significantly higher ring-stage survival of the parasite *in vitro* and delayed parasite clearance *in vivo*, as reported previously. Although AL remains highly efficacious in Africa including Kenya, it is also associated with selection of SNPs in *Plasmodium falciparum* chloroquine resistance transporter gene (pfcrt) and *P. falciparum* multidrug resistance gene 1 (pfmdr1) in re-infections. The genotype associated with re-infection is K76 in pfcrt and N86, 184F and D1246 (NFD) in pfmdr1; K+NFD haplotype. In Africa however, data suggests K+NFD haplotype is selected for by AL, though no evidence linking this to artemisinin resistance. This study aims to investigate genetic markers for any polymorphisms found in western Kenya parasites associated with changes seen in clinical phenotype. K13 full gene was sequenced using previously published and designed primers. For Pfmdr1 gene, regions covering SNP 86, 184 and 1246 were amplified while for Pfcrt we amplified regions flanking the 72-76 haplotype region. Ring stage assay was performed and survival rates calculated. Pfmdr1 gene showed 95.7% wild, 58% mutant and 81.5% wild at codons 86, 184 and 1246 respectively. For haplotypes, K+NFD had highest frequency of 48.7%. K13 Full gene analysis showed 61 (84.7%) had non-synonymous SNPs in >5 locations out of which K189T dominated with highest frequency of (20.2%). Other SNPs include: Y493I (1.2%), A578S (1.9%) reported elsewhere while E433K (6), D464N (6), F483L (5) are uniquely found in western Kenya parasites (yet to be validated). Also, no variations in the number of a microsatellite repeat (ATA) corresponding to amino acid positions 137-142 of K13 was observed. No direct correlation seen between the mutations and the survival rates >10. It is evident that genetic determinant for higher survival rates to ACTs in African parasites is different compared to SEA parasites. K13 mutations described here including K189T are not linked to artemisinin resistance, as reported previously.

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HEPATOMEGALY IN ACUTE FALCIPARUM MALARIA IN NIGERIAN CHILDREN: BEFORE, DURING AND AFTER ARTEMISININ-BASED COMBINATION TREATMENTS

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Hepatomegaly or splenomegaly is common in childhood acute falciparum malaria but there is little evaluation of the risk factors, temporal changes and the disposition kinetics of the malaria-associated hepatomegaly following artemisinin-based combination treatments (ACTs). Liver and spleen enlargement below costal margin was detected by abdominal palpation and percussion and the liver size measured over a 6-week period. Changes in liver size was characterized using the rate of decrease or increase in size. Kinetics of the disposition of liver enlargement was evaluated using a non-compartment model. At presentation, hepatomegaly was significantly more common than splenomegaly in acutely malarious children (224 *versus* 129 of 786 children, $P < 0.0001$).

In a multivariate analysis, an age <5 years (adjusted odd ratio [AOR] = 3.1, 95%CI 2.2-4.6, P < 0.0001), duration of illness >2 days (AOR = 1.5, 95%CI 1.1-2.2, P = 0.02), core temperature >39ºC (AOR = 1.5, 95%CI 1.0-2.2, P = 0.03) and haematocrit <30% (AOR = 2.1, 95%CI 1.2-3.6, P = 0.01) were independent predictors of hepatomegaly or splenomegaly at presentation. The commonest temporal change following ACTs was ultra-rapid complete regression of hepatomegaly in over 93% of the children. Overall, mean hepatomegaly regression time (HRT) was 2.9 days (95%CI 1.9 – 3.8) and it was similar in artesunate-amodiaquine- and artemether-lumefantrine-treated children. Declines in hepatomegaly were monoexponential with overall estimated half-time ($t_{½hep}$) of 0.2 day (95%CI 0.2-0.3). HRT correlated significantly with $t_{½hep}$ (P < 0.0001). Bland-Altman analysis of 9.5 and 10 multiples of $t_{½hep}$ and HRT showed narrow limit of agreement with insignificant bias (P ≥ 0.06) suggesting both can be used interchangeably in the same patients. Young, febrile and anaemic malarious children with duration of illness >2 days, are at significant risk of hepatomegaly or splenomegaly at presentation. In acute childhood falciparum malaria, regression of hepatomegaly is rapid and is a first-order process following ACTs.

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GROWTH ADVANTAGE OF WILD-TYPE OVER DRUG RESISTANT *PLASMODIUM FALCIPARUM* GENOTYPES AMONG ASYMPTOMATIC CARRIERS IN ABSENCE OF THERAPY

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A large set of data, field and animal models, demonstrated a compromised fitness of drug-resistant malaria parasites in absence of chemotherapy. Here we extended the above observations, and quantitatively monitored drug resistant and sensitive parasite genotypes, within asymptomatic *Plasmodium falciparum* carriers, in absence of therapy, and determine their relative growth and ability to produce gametocytes. We test the prediction that drug resistant genotypes in absence of therapy are constrained by competition with co-infecting wild-type clones to maintaining low gametocyte production. We recruited 123 patients, in a seasonal malaria setting, where transmission occurs over a short period, and the rest of the year remains transmission-free. The patients were initially treated, and then monitored monthly throughout the long dry season. Relative abundance (RA) of wild- versus mutant genotypes of pfcrt and pfmdr-1 was determined using qPCR. Parasite and gametocyte densities were quantified by qPCR of 18s rRNA and RT-qPCR of pfs25 and pfs230, respectively. The densities of the mutant genotype of pfcrt and pfmdr-1 were high at enrolment and post treatment, however it decreased steadily over the dry season. The RA of wild-type of both genes increased substantially over the long dry and transmission-free period (pfcrt, p=0.009738; pfmdr-1, p=0.006867). Gametocytes density negatively correlated with the RA of wild-type pfcrt-CVMNK (p = 0.03044) but not with the wild-type pfmdr-1 86N (p= 0.06512). We provide the first direct evidence for within host growth advantage of wild-type strains of *P. falciparum*, in a therapy-free environment among asymptomatic carriers. Thus, in areas of seasonal transmission, the dry season can play an important role in harnessing susceptible parasites.

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ACTIVE MONITORING OF PHARMACOVIGILANCE AT COMMUNITY LEVEL DURING THE SEASONAL MALARIA CHEMOPREVENTION CAMPAIGN IN THE HEALTH DISTRICT KOLDA SENEGAL, 2015

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The scaling up of seasonal malaria chemoprevention campaign (SMC) began in Senegal in 2013 in four health districts with an extension in 2014 to cover four regions and over 600 000 children aged 3 months to 10 years. It is made in the form of local distribution campaigns / free mass administration of drugs Sulfadoxine-pyrimethamine (SP) + Amodiaquine (AQ) by a door to door strategy relying on the community network. The regions of Kedougou, Tambacounda, Kolda and Sedhiou meet the WHO criteria for eligibility. While the drugs used in SMC are generally considered safe and effective, they can cause adverse events that may be minor, moderate, or in very rare cases, severe. Spontaneous reporting is essential but not sufficient to assess any adverse effects related to the use of a drug. Its need to develop a community-based active pharmacovigilance through a cohort followed for SMC. The overall objective was to assess the ability of a strategy to strengthen the pharmacovigilance system and improve the reporting rate of adverse events in the context of SMC. The study took place in the health district of Kolda in southern Senegal during the season 2015 SMC for a total of three rounds (August, September and October) for 10,000 children benefiting SMC in three health posts district. In the same district, three other health posts targeting the same number of children were selected as controls. The side effects filings were received by the NMCP and processed by the pharmacovigilance center. In total 829 adverse events were reported for 743 consultations. More than half of the symptoms reported (591) were gastrointestinal disorders (71%). Other common symptoms are fever (24%). Accountability side effects made by the pharmacovigilance center and approved by the technical committee of experts shows 48% possible and probable for 28%.These results show a clear improvement compared to routine pharmacovigilance system implemented in other health districts. All results must be sent to Uppsala center UMC / WHO by vigiflow.

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HOT AND STICKY: THE EFFECT OF TROPICAL CONDITIONS ON THE STABILITY OF ARTEMISININ-BASED COMBINATIONS THERAPIES

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Poor-quality medicines, including falsified, substandard and degraded drugs, pose serious health concerns in malaria endemic countries as they contribute to the rise in drug resistance, can kill patients, and increase the public's mistrust of health systems. Substandard drugs are defined as those that contain either less or more than the acceptable dose (compliance with pharmacopeia tolerance limits) of stated active pharmaceutical ingredients (SAPIs) resulting from poor manufacturing practices, while degraded drugs are good quality formulations that have degraded on storage in the presence of heat, light and humidity. Guidelines are lacking distinguishing between substandard and degraded drugs in terms of markers of degradation. 'Forced degradation' was carried out on three common artemisinin-based combination therapy (ACT) brands and analytical methodology was developed to facilitate the classification of degraded drugs. This methodology was applied to ACTs purchased in Enugu, Nigeria that had been classified to be substandard (206 samples) and 18% of these were found to contain degradation products. We previously conducted a large-scale 'natural ageing' study (2,880 samples

of Coartem® and Winthrop®) to evaluate the long-term stability of ACTs in tropical climates, on-site in Ghana and in a stability chamber in London. Samples were aged in the presence and absence of light and removed from each site at regular intervals to measure loss of SAPIs over time as well as detect products of degradation. Loss of SAPIs in these naturally aged samples was 0 to 7% over 3 years (~12 months beyond expiry) with low levels of degradation products detected. ACTs that were found to be stable in tropical climates for periods up to and beyond their expiry dates were from WHO prequalified manufacturers, while those found to contain degradation products were from non-WHO prequalified manufacturers. Hence, presence of insufficient SAPIs, together with detection of degradation products, can be used to classify drugs as degraded.

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READMISSION TO HOSPITAL FOR WORSENED ANAEMIA IN MOZAMBICAN CHILDREN TREATED WITH INTRAVENOUS ARTESUNATE

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Readmission to hospital for worsened anaemia in Mozambican children treated with intravenous artesunate Parenteral artesunate is recommended as first-line therapy for severe malaria. While its efficacy is firmly established, data on safety are still incomplete and scarce among African children. We aim to assess and compare delayed (newly incident or worsening) anaemia rates in the first 30 days after parenteral treatment with Quinine or artesunate, after its introduction as first line policy. We also aim to identify risk factors associated to delayed anemia following artesunate iv treatment. We conduct a retrospective analysis for the period 2001-2015 using the outpatient and inpatient morbidity databases, and linking them with the demographic surveillance system ongoing in the Manhiça district, in Southern Mozambique. Recurrent hospital admissions or outpatient visits after a documented parenteral treatment for malaria will be analysed to determine whether the use of parenteral artesunate is associated with an increased risk of anaemia. Comparison of trends of this phenomenon (before and after the year when the switch from quinine to artesunate in first line policy occurred), in addition to the evaluation of other potential confounding factors, will allow an inference of the possible attribution to artesunate. We will present the results of this analysis, reviewing over 50,000 admissions during the study period.

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IMPROVING UPTAKE OF IPTP IN UGANDA THROUGH TEXT MESSAGING HEALTH WORKERS

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Malaria in pregnancy poses a risk to mother and child. The World Health Organization recommends intermittent preventive treatment in pregnancy (IPTp) for the prevention and control of malaria in pregnancy, typically provided through the focused antenatal care (ANC) package. Coverage of at least two doses of IPTp remains low in most countries implementing IPTp, despite generally high ANC attendance. Qualitative formative research conducted in 2013/14 concluded that supply-side issues are likely to account for the majority of missed opportunities for the provision of IPTp in Uganda. In particular, health workers' knowledge of IPTp guidelines was poor. To address this barrier, a pilot intervention was implemented in West Nile in 2015. This involved provision of classroom-based malaria in pregnancy training to selected health workers in two districts (n=24 per district). In one district only, all health workers involved in ANC provision (n=49) subsequently received 25 text messages reinforcing the training content. The study used a mixed-methods design to determine the impact of text messaging: i) a multiple choice knowledge questionnaire administered immediately after training (n=90) and six months after training (n=89), ii) calculation of IPTp coverage in participating health facilities (n=16) over six months pre- and post-training and iii) four focus group discussions with health workers and three in-depth interviews with district officials. Health workers who had received text messages in addition to training demonstrated better knowledge of malaria in pregnancy six months post-training compared to health workers receiving training only. IPTp coverage was also higher in facilities where health workers received text messages. Complementing classroom-based malaria in pregnancy training with text messages has the potential to improve health worker knowledge of and adherence to guidelines. Text messaging is inexpensive, well-received by health workers and does not disrupt service provision. This approach has potential applications to health worker training on other topics.

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EFFICACY OF THREE ARTEMISININ-BASED COMBINATION THERAPIES IN ANGOLAN CHILDREN WITH *PLASMODIUM FALCIPARUM* INFECTION, 2015

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Antimalarial resistance monitoring in Angola has recently garnered increased attention. Therapeutic efficacy studies (TES) from 2011-2013 in Luanda and from 2013 in Zaire Province showed efficacy of artemether-lumefantrine (AL) approaching the 90% threshold identified by the World Health Organization for artemisinin-based combination therapies (ACTs). In addition, a controversial case of malaria unresponsive to artemisinins was reported in a patient infected in Lunda Sul Province in 2013. In January-June 2015, investigators monitored the clinical and parasitological response of children with uncomplicated *Plasmodium falciparum* infection treated with one of three ACTs: AL, artesunate-amodiaquine (ASAQ), or dihydroartemisinin-piperaquine (DP). The study comprised two treatment arms in each of three provinces: Benguela (AL and ASAQ), Zaire (AL and DP), and Lunda Sul (ASAQ and DP). Participants were followed for 28 (ASAQ and AL) and 42 (DP) days. Samples from treatment failures were analyzed for molecular markers of resistance for artemisinin (K13) and lumefantrine (pfmdr1). A total of 475 children reached a study endpoint. Fifty-five treatment failures were observed: 4 early treatment failures, 41 reinfections, and 10 recrudescences. Excluding reinfections, the microsatellite-corrected efficacy at day 28 was 96.3% (95% CI: 91-100) for the AL arm in Benguela, 99.9% (95-100) for ASAQ in Benguela, 88.1% (81-95) for the AL arm in Zaire, and 100% for ASAQ in Lunda Sul. For DP, the corrected efficacy at day 42 was 98.8% (96-100) in Zaire and 100% in Lunda Sul. All treatment failures were wildtype for K13, but all AL treatment failures had pfmdr1 haplotypes associated with decreased lumefantrine susceptibility. The results suggest a parasite population sensitive to artemisinin derivatives in these three provinces. No evidence was found to corroborate the specific allegation of artemisinin resistance in Lunda Sul. The continued low efficacy of AL in Zaire might be due

to decreased susceptibility to lumefantrine, and further monitoring, particularly including measurement of lumefantrine blood levels during TES, is recommended.

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ACCELERATING THE DISCOVERY OF TRANSMISSION-BLOCKING DRUGS: HT SCREENING WITH A NOVEL *PLASMODIUM FALCIPARUM* FUNCTIONAL GAMETOCYTE ASSAY

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The last decade has witnessed unprecedented progress in reducing the incidence of *Plasmodium falciparum* malaria, especially in Africa where the burden of disease is greatest. Nevertheless, in order to achieve the ultimate goal of eradicating malaria, more effective tools will be required. These include efficacious vaccines and innovative antimalarial drugs with novel mechanisms of action displaying not only efficacy against resistant parasites but also transmission blocking potential. *P. falciparum* mature gametocytes are the parasite forms responsible for malaria transmission, thus our objective was the discovery of drugs with a novel mechanism of action, active against these stages, either by killing or functionally preventing their maturation to mosquito stages. For this purpose, we have developed a phenotypic HTS assay, which assesses the functionality and viability of *P. falciparum* stage V gametocytes by measuring the formation of female gametes. In this work, we present the screening of GSK compound collections. Over 400 hits were identified after a primary screening at 2μM and a subset of approximately 100 compounds representative of new chemical diversity were prioritized for further profiling. A selection of hits was assessed *ex vivo* in the standard membrane feeding assay and demonstrated complete transmission blockage. This new set of compounds may serve as starting points for future drug discovery programs as well as tool compounds for identifying new modes of action involved in malaria transmission.

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NEW ANTIMALARIAL FAMILY "RESISTANT TO RESISTANCE" DEVELOPMENT

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Malaria continues to be a major global disease still causing impermissible number of deaths. The effectiveness of current antimalarial therapy is under continuous threat through the spread of resistance. Consequently, there is an urgent need to replace those drugs compromised by resistance, as well as identifying potential novel therapies that offer significant advantages over the current standard of care. As result of the whole cell phenotypic screen conducted by GSK to identify TCAMS set of compounds, a number of lead optimization programs were initiated. One of them, pyrazine family, was progressed because the chemical novelty displaying exciting opportunities as antimalarial. The program led to the identification of balanced molecules with appealing biological profile. Pyrazine molecules demonstrated relevant activities against intra-erythrocytic asexual *Plasmodium* stages including multiple MDR laboratory adapted strains and clinical isolates. Pyrazines displayed a desirable rapid *in vitro* killing profile. This activity is accompanied by an oral efficacy characterized by a rapid parasite clearance in the *P. falciparum* mouse model. This rapid antimalarial activity is expected to maximize efficacy, achieving fast clinical resolution, and minimizing the in-patient window of opportunity for resistant parasite selection and dissemination. Notably, the series displayed an extremely low propensity to select resistance *in vitro*. The biological profile that the series offers seems to be associated to an unique mode of action not related to any of the known mechanisms tested, hence offering differentiation against all other antimalarials. The development of differentiated MoA antimalarials is of paramount importance to be able to develop de novo combination treatments, thereby ultimately avoiding the emergence of resistance. The overall properties of pyrazines constitutes a promising profile justifying further development and makes these assets suitable partners for any future combination treatment.

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IN VIVO STUDIES OF FITNESS OF DRUG RESISTANT *PLASMODIUM FALCIPARUM* DHFR MUTANTS USING *PLASMODIUM BERGHEI* TRANSGENIC PARASITES

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Drug resistance has hindered control efforts for malaria, where mutations to the target dihydrofolate reductase (DHFR) enzyme are responsible for resistance to pyrimethamine. Nonetheless, antifolates are key components of ACT's and used for intermittent preventive treatment in pregnancy. Understanding parasite fitness evolution is important for transmission. There is a lack of information about the cost of drug resistance mutations on parasite fitness, or the ability of resistant parasites to compete with sensitive strains and persist in the field. We studied how DHFR mutations under drug pressure influences survival of resistant alleles. We conducted competition experiments between *Plasmodium berghei* parasites in which endogenous dhfr was modified by gene replacement, with *P. falciparum* single (108 {1M}), double (59 and 108 {2M}) and triple (59, 108 and 164 {3M}) mutants. For each parasite line created, GFP and cherry RFP were used as reporters to aid parasite enumeration via flow cytometric analysis. All single infection and competition experiments were initiated with 1x106 parasites. Infections were followed up, with the treated groups administered 10-mg/kg pyrimethamine for 3 days, after which, parasitemia was assessed via counting of fluorescent parasites by a flow cytometer. In all experiments (both treated and non treated groups), single infection parasites grew better in mixed infections (competition). For all mutant lines in competition experiments, higher resistant parasites grew better in mixed infections when competing against lesser resistant mutants. 2M mutants grew better in single infections with drugs than without drugs. In all competition experiments 3M mutants grew better with or without drugs than other mutants. These findings support a model of parasite release and facilitation, whereby under drug pressure highly resistant parasites do better than less resistant parasites. Recognizing the costs of fitness will aid the development of optimal guidelines for the treatment and prevention of malaria.

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EVALUATION OF THE PERFORMANCE OF SD BIOLINE MALARIA AG *PF/PV* AND CARESTART™ MALARIA *PF/PV* COMBO TESTS FOR THE DIAGNOSIS OF MALARIA IN TWO MALARIOUS AREAS IN CENTRAL ETHIOPIA

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Early and accurate diagnosis of malaria followed by prompt treatment reduces morbidity and mortality in endemic regions. Presumptive

treatment of malaria is widely practiced where microscopy or rapid diagnostic tests are not readily available. Introduction of rapid diagnostic tests (RDTs) for the treatment of malaria in many low-resource settings need evaluation of their performance. This study evaluated the performance of two RDTs in two health centers from November-December, 2014 in Adam and Amaya. Oromia, Ethiopia. This study was undertaken to evaluate the diagnostic performance of SD BIOLINE malaria Ag *Pf/Pv* and CareStart™ malaria *Pf/Pv* Combo test relative to microscopy for the diagnosis of *P. falciparum* and *P. vivax* malaria in Ethiopia. In this cross-sectional study, patients who had malaria symptoms and visited two health facilities in Oromia Region were recruited. Thin and thick blood smears were prepared from finger prick and stained by 10% Giemsa. Microscopic examination was done under 100x magnifications for *Plasmodium* species identification and determination of parasitaemia. The two RDTs were performed as per the manufacturers instructionsA total of 547 febrile patients were diagnosed, of which 127 were microscopy positive for *Pf* (n=38) and *Pv* (n=85). The sensitivity, specificity, positive and negative predictive value of SD BIOLINE malaria Ag *Pf/Pv* test were 92.1%, 99.1%, 95.9% and 98.2%, respectively; and for CareStart™ malaria *Pf/Pv* Combo tests were 94.5%, 99.6%, 98.4% and 99.6%, respectively. In conclusion, the diagnostic performance of SD BIOLINE malaria Ag *Pf/Pv* test and CareStart™ malaria *Pf/Pv* Combo test were very good with respect to malaria microscopy.

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THE EFFECTS OF MALARIA SPECIES-SPECIFIC TREATMENT POLICY ON CASE MANAGEMENT AND DYNAMICS OF RESISTANCE MARKERS IN ETHIOPIA

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In Ethiopia, *Plasmodium falciparum* and *P. vivax* are co-endemic and virtually responsible for 60 and 40% of all malaria cases, respectively. Artemether-lumefantrine and CQ are first-line treatments for uncomplicated *P. falciparum* and *P. vivax* infections, respectively. The change from one drug to another, following the emergence of drug resistance, would alter the dynamics of sensitive and resistant parasite haplotype. Accurate diagnosis is, therefore, crucial for appropriate prescription of the correct drugs. A total of 1,094 patients were screened for malaria using microscopy and RDTs. Sets of microscopy and RDT-positive and negative samples were tested for confirmation using PCR. Anti-malarial drugs were prescribed for 9.3 and 20.0% microscopy- and RDT-based parasite negative patients, respectively, as confirmed by PCR. PCR detected the presence of *P. falciparum* DNA in 19.2% of microscopy- and RDT-negative samples. Of microscopy-positive *P. vivax* infections, 63.2% were proved *vivax* malaria by PCR. Of microscopy-positive *P. falciparum* infections, 71.4% were proved *falciparum* malaria by PCR. Of RDT-positive *P. falciparum* infections, PCR proved in 62.7% of them. Of RDT-positive *P. vivax* infections, 77.3% were proved vivax malaria by PCR. All parasites were carrying the *pfcrt* K76T mutant variants. The prevalence of parasites with the wildtype codon *pmdr*1 Y86N was 76.7%. Microscopy and RDT were insufficient at low density parasitaemia and hence misdiagnosis was significant. The use of molecular methods appropriate at field setting would help avoid over-prescription and under-diagnosis of malaria infections. While the presence of the mutant variant of *pfcrt* in the Ethiopian samples can be explained by continued use of chloroquine in Ethiopia for treatment of *P. vivax*, the selection of wild type *pfmdr*1 could be a consequence of using ACT for treatment of *P. falciparum.*

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PARASITOLOGIC CORRELATES OF *PLASMODIUM OVALE WALLIKERI* AND *PLASMODIUM OVALE CURTISI* INFECTION

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Plasmodium ovale is one species of human malaria that is prevalent in West Africa and can be challenging to diagnose due to clinically mild disease and low parasite burden. Consequently, conventional diagnostic tools, such as microscopy of blood films and rapid antigen-based diagnostic tests, have limited performance in detecting *P. ovale* infection. Furthermore, two genetically distinct sub-species of *P. ovale* exist: *P. ovale* curtisi (Poc) and *P. ovale* wallikeri (Pow). At present, it is unknown if the sub-species causing infection may affect clinical presentation or performance of malaria diagnostic tests. Therefore, we sought to investigate if parasite burden, morphological features, and pan-aldolase antigen-positivity differ between Poc and Pow. 49 *P. ovale*-positive, whole-blood specimens were identified from our malaria biobank, and DNA was extracted. Parasitemia and pan-aldolase antigen-positivity that were reported upon initial processing were obtained for analysis. Real-time PCR (qPCR) assays were conducted to confirm microscopy species identification, and quantify 18S rRNA gene copy number. Endpoint PCR of target regions and Sanger sequencing were conducted, and sub-species was determined by analyzing the 18S rRNA sequence. We compared reported parasitemia, parasite morphology, pan-aldolase antigen-positivity, and 18S gene copy number between the two sub-species. We identified 22 Poc and 27 Pow. There were no statistically significant differences between the two sub-species by parasitemia, 18S rRNA gene copy number, or pan-aldolase antigen-positivity. When comparing morphological features, we noted that all 8 *P. ovale* parasites without Schuffner's stippling were Pow while all Poc parasites had this feature (p=0.02). Poc and Pow do not differ significantly by parasite burden, although a lack of discernible Schuffner's stippling may be a feature specific to Pow. This is a novel finding, considering that the sub-species have been previously distinguished by their genotype alone.

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FALSE-NEGATIVE MALARIA RAPID DIAGNOSTIC TESTS IN RWANDA: IMPACT OF *PLASMODIUM FALCIPARUM* ISOLATES LACKING HRP2 AND IMPROVED MALARIA CONTROL

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Rapid Diagnostic Tests (RDTs) have become the focal point of the global approach to malaria control and are often used to determine whether persons with fever, chills or other symptoms of malaria are treated with antimalarials. Because false-negative RDTs can delay or prevent the effective treatment of this potentially fatal disease, it is essential to examine the factors that affect RDT performance. Recently, *Plasmodium falciparum* isolates lacking the *hrp2* gene have been identified as a cause of false-negative RDTs. However, despite the importance of this issue, there is a paucity of data on the frequency of these isolates. This study examined RDT sensitivity at sites with varying intensities of malaria transmission in Rwanda and used PCR to determine whether *hrp2* deletions were responsible for false-negative HRP2-based RDT results. Between April 2014 and April 2015, the study enrolled 9,219 symptomatic patients from 3 health centers in Rwanda and compared RDT results to microscopy for *Plasmodium* species as the gold standard. The overall slide positivity rates (SPR) were 53% at Rukara, 35% at Kibirizi and 10% at

Busogo. RDT sensitivity varied by month and site and was highest (94% [95% CI 92-95%.]) at Rukara, the site with the highest SPR. At Kibirizi, a site targeted by pre-elimination activities during this study, RDT sensitivity declined from 88% to 67% as the monthly SPR fell from 46% to 3%. For samples that were positive by microscopy but negative by RDT, PCR was performed to test for *Plasmodium* DNA and confirm the presence or absence of the *hrp2* gene. PCR analysis identified a total of 34 infections that were positive by PCR for *P. falciparum* but negative by RDT and PCR for *hrp2* (consistent with deletion of the *hrp2* gene). To the authors' knowledge, this is the first report of circulating *P. falciparum* isolates lacking *hrp2* in East Africa. However, this is not the first time that a decline in RDT sensitivity occurred following a decline in malaria transmission. Further investigation is warranted to assess the factors driving the decline in RDT sensitivity as malaria control improves.

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SNP-LAMP POINT OF CARE TEST FOR THE DETECTION OF ARTEMISININ-RESISTANCE IN THE FIELD

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Cases of artemisinin resistant malaria are rapidly increasing in South-East Asia. Even in some part of Cambodia, the proportion of the slow clearing parasites is now above 75%. Several mutations in the K13 propeller gene have been associated with 91.8% of slow-clearing parasites. Of these drug resistant mutations, C580Y and R539T constituted 85% of the resistant population in Cambodia. Recently,the C580Y mutation was also reported from Guyana, a South American country but not Africa. Monitoring resistance in the field site is important for the treatment and surveillance of resistant clones. Current methods to evaluate artemisinin resistance such as *in vitro* culture methods and DNA sequencing are time-consuming, reagent intensive, expensive and not applicable to the field setting. Point of care detection of K13 mutations will allow for correct antimalarial treatment choices as well as enhance surveillance. Loop mediated amplification (LAMP) has proven to be field adaptable in our hands. We now modify LAMP to detect the C580Y and R539T SNP. SNP-LAMP is based on the differential binding of amplification primers. Our strategy was to validate SNP-LAMP on bona fide resistant clones from Cambodia. Input template DNA and enzyme concentrations were varied to optimize amplificationof the mutant strain while not amplifying the wild type. Preliminary data is presented on the K13 SNP-LAMP assay for control strains, clinical isolates, and non- falciparum controls including *Plasmodium vivax*, *P. ovale* and *P. malariae*. Our data suggest the mutant K13 propeller gene can be specifically amplified within a range of 1000-10000 parasites/µL.Specificity is lost if parasitaemia goes over 10000/µL. Dilution of the high copy number sample can maintain the accuracy of the diagnosis. Further work is going on improve the minimum and maximum limit of detection by altering different sequences of loop primers.

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ASSESSMENT OF LOOP MEDIATED ISOTHERMAL DNA AMPLIFICATION (LAMP) METHOD FOR ASYMPTOMATIC MALARIA SCREENING IN THE PERUVIAN AMAZON SETTINGS

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In the Peruvian Amazon, asymptomatic malaria infections, with low parasite density, are common and has impact on maintaining malaria transmission. Within this context, molecular diagnostic tests would be more efficient than microscopy or RDTs to identify these cases; however, it requires well trained users and special equipment. LAMP is expected to be a point-of-care method that could be applied in rural communities with basic facilities and also an alternative for population screening aiming malaria elimination. In this work, we assessed the performance of LAMP to detect asymptomatic malaria infections compared with microscopy and validated by three different PCR protocols: nested PCR (nPCR), qPCR base on mitochondrial genome (qPCR-Pgmt) and qPCR base on 18S rRNA gene (qPCR-18S). 1173 subjects from eight communities along Alto Nanay river were screened by LAMP and microscopy and all asymptomatic individuals above 3 years old were enrolled. 58 (4.9%) were positive by microscopy and 259 (22.1%) were positive by LAMP (34 *Plasmodium falciparum* and 225 no-falciparum). All LAMP positive samples, 30% of negative samples and 7 positive controls with different parasitaemia were tested to evaluate LAMP sensitivity and specificity. Stratified analysis by parasitaemia level between LAMP and PCR showed a sensitivity of 100%, 94.8% and 93.1% against nPCR, qPCR-Pgmt and qPCR-18S, respectively, when parasitaemia was higher than 1 parasite/µL. However, with parasitaemias bellow 1 parasite/µL, LAMP sensitivity decrease to 77.8%, 86.6% and 69.9% against nPCR, qPCR-Pgmt and qPCR-18S, respectively. Our results suggest that LAMP is a good alternative for a point of care diagnostic and population screening, but its performance decays with samples with very low parasitaemia. To move towards malaria elimination in settings with very low transmission, LAMP performance should be improved, and the development of new tests capable of identifying very low parasite densities is required.

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VALIDATION OF DRUG SELLERS' MALARIA RAPID DIAGNOSTIC TESTS (MRDTS) RESULT RECORD AND FIELD MALARIA RDT PERFORMANCE AGAINST PCR ANALYSIS OF DUPLICATE BLOOD SAMPLES FROM UNDER-FIVE CHILDREN IN SOUTHWESTERN UGANDA

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Currently, the WHO recommends that every case of suspected malaria be confirmed by a diagnostic test. Use of malaria Rapid Diagnostic Tests (mRDTs) has been emphasized in communities and private drug outlets where more than 50% of fever case management occurs in malaria endemic LMICs. Due to limitations in mRDT sensitivity for efficient detection of low-density parasitemia or sub-microscopic infections, nucleic acid amplification techniques (NAATs) have been encouraged for epidemiological research and surveys to map sub-microscopic malaria infections in low-transmission settings such as South Western Uganda. Operator error, transport and storage conditions can also cause false negatives. On this basis, we are comparing test results from mRDT done by drug sellers in field conditions on under-five children in South Western Uganda against PCR test. A duplicate blood sample is picked using Whatman FTE card for additional test comparisons. Methods Duplicate samples from under-five children are collected using CareStartTM HRP2 Pf RDT cassettes and WhatmanTM 3MM filter paper at drug shops participating in the on-going AXEX study in cross-sectional study design. A total of 203 samples have been collected and analysis is ongoing at the Molecular Biology Lab at Makerere University. Proficiency testing on the PCR method was done. The method includes extracting *Plasmodium* DNA from blood spot collected on Whatman FTE card and mRDT cassette using Chelex method and Qiagen Blood DNA kit following manufacturer's instructions. The quantity and quality of the extracted DNA is assessed using a Nano Spectrophotometer and QIAexcel automated capillary electrophoresis. Results Results to be presented will include field mRDT performance when test is done by drug seller against PCR analysis. Sensitivity, specificity, positive and negative predictive values will be reported. Agreement between the two tests will also be calculated and evaluated using the kappa statistic. In conclusion, this study will provide additional empirical information on who to deploy mRDT alongside NAATs in screening and surveillance of malaria during country elimination efforts.

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HEALTH WORKER AND POLICY MAKER PERSPECTIVES ON USE OF INTRAMUSCULAR ARTESUNATE FOR TREATMENT OF SEVERE MALARIA AT HEALTH POSTS IN ETHIOPIA

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World Health Organization (WHO) recommends intravenous (IV) and intramuscular (IM) artesunate (AS) for management of severe malaria. The current national malaria treatment guidelines do not provide for use of IM AS at health posts (manned by health extension workers (HEWs)). Although WHO recommends use of pre-referral intrarectal artesunate, there are no WHO prequalified suppliers of intrarectal artesunate, and its use is limited to children under 6 years of age. We assessed the perspectives of health workers, and policy makers on the use of IM AS as a pre-referral and definitive treatment for severe malaria at health post level in Ethiopia. A qualitative exploratory study that employed in-depth interviews with 101 health workers from 60 health facilities, the Federal Ministry of Health, and development partners. All respondents were either health workers involved in the treatment of malaria, or in formulation of malaria policy. An interview guide was used. Data transcripts were translated into English, uploaded into Atlas.ti7 software, and coded. Thematic content analysis was employed. Key findings from this study are: (1) Provision of IM AS as pre-referral and definitive treatment for severe malaria at health posts could be lifesaving; (2) With adequate training, and provision of facilities including beds, health posts can provide definitive treatment for severe malaria using IM AS where referral is delayed or not possible; (3) Health workers at health centers and hospitals frequently use the IV route because it allows for co-administration of other drugs, but they find the IM route easier and propose it for HEWs; (4) The reasons commonly cited against the management of severe malaria using IM AS at community level were: Lack of capacity to manage complications and fear of irrational drug use; (5) use of IM AS at health post level will require evidence on safety and feasibility before policy shift. IM AS could be used at health posts to provide life-saving pre-referral, or definitive treatment when referral is delayed or not possible. Evidence on safety and feasibility of its use by HEWs is needed before policy change.

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EVOLUTION AND SPREAD OF "STEALTH" *PFHRP2* DELETIONS IN *PLASMODIUM FALCIPARUM* IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Deletions of the *Plasmodium falciparum hrp2* (*pfhrp2)* gene cause false-negative rapid diagnostic test (RDT) results and have been sporadically reported but never investigated systematically on a national population level. RDTs are a cornerstone of modern malaria control efforts; understanding the evolution of *pfhrp2* deletions is essential for ensuring their continued effectiveness. Using a nationally representative cross-sectional study of 7,137 children in the Democratic Republic of Congo, we investigated whether *P. falciparum* parasites with false-negative RDT results harbored deletions of *pfhrp2*. We employed polymerase chain reaction assays to identify *pfhrp2*-deleted parasites among those with false-negative RDTs and to examine microsatellite regions flanking the *pfhrp2* gene for population genetic analyses. We found that 4.3% (n = 117) of all *P. falciparum* infections country-wide were due to *pfhrp2*-deleted mutants, representing 14.9% of 783 parasites with false-negative RDT results. Bayesian spatial analyses identified two geographical clusters with significantly higher proportions of parasites harboring *pfhrp2* deletions. Population genetic analysis of these clusters revealed significant genetic differentiation between wild-type and *pfhrp2*-deleted parasite populations (G_{ST} = 0.021, $p \leq 0.00001$). In conclusion, *Pfhrp2*-deleted *P. falciparum* is a common cause of false-negative RDTs in the DRC. Use of RDTs as indicators for treatment may be exerting evolutionary pressure favoring the spread of "stealth" parasites, resistant to detection by currently used RDTs. To our knowledge, this is the first report of an outbreak of a pathogen that has mutated to elude detection by a diagnostic test.

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INTRODUCTION OF COMPETENCY BASED SELECTION CRITERIA FOR WHO EXTERNAL COMPETENCY ASSESSMENT FOR MALARIA MICROSCOPY

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In 2015 MalariaCare introduced a new selection criterion to the WHO External Competency Assessment for Malaria Microscopists (ECAMM) to determine the best-qualified candidates among those who met course entry requirements. We describe a competency-based modification to existing ECAMM course entry requirements that can be used to identify the best-qualified candidates for participation in WHO ECAMM courses. Pools of candidates were screened using existing WHO ECAMM course entry requirements. A second selection criterion was added based on satisfactory performance from a five-day pre-ECAMM refresher training course. Of the 119 participants included in the final WHO ECAMM data set, 103 (86.6%) were assessed prior to 2015 and did not participate in a pre-ECAMM course; however, 16 (13.4%) microscopists assessed in 2015 participated in a pre-ECAMM course and were selected for advancement to WHO ECAMM courses based on attainment of prescribed competency levels. Post-test pass rates for WHO ECAMM course components among microscopists not participating in pre-ECAMM courses were 82.5% for parasite detection (mean score = 89.8%), 26.2% for species identification (mean score = 62.5%), and 43.7% for parasite quantitation (mean score = 34.8%). Among participants who were subjected to the revised selection criteria, post-test pass rates for all 3 WHO ECAMM course components were 100.0%. Mean post-test scores within this participant pool were 97.6% (parasite detection), 90.2% (species ID), and 60.8% (parasite quant).Participants attending WHO ECAMM courses before 2015 were 3.7 times less likely to attain WHO certification, whereas all participants from the 2015 participant pool attained WHO certification based on their accreditation levels. WHO ECAMM course outcome (certification vs. non-certification) was not independent of participant selection criteria type, c^2 (1, N=119) = 35.87, p<0.0001. To identify the best qualified participants, our results suggest that course administrators may consider a second competency-based selection criterion based on satisfactory completion of a five-day pre-ECAMM refresher-training course.

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METHODS FOR IMPROVED NUCLEIC ACID-BASED DETECTION OF INTRAERYTHROCYTIC *PLASMODIUM* AND BABESIA PARASITES

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We have developed a novel nanoparticle-based sandwich hybridization assay (SHA) for the detection of *Plasmodium falciparum* (Pf) and *Babesia microti* (Bm) parasites without the need for amplification of target sequences in genomic DNA. A uniquely identifiable "barcoded" magnetic microbead and biotinylated silica nanoparticle are conjugated to either Pf or Bm specific 30-mer oligonucleotides corresponding to sequences of the 18S ribosomal gene. Parasite burden can then be quantified and analyzed upon the binding of an Avidin-PE fluorophore to the target capture complexes via a Bio-Plex 200 instrument. Probit analysis of 1 mL of parasite-spiked human blood revealed 95% detection thresholds of 62 and 122 p/mL for the Pf and Bm 18S bead sets, respectively. To further enhance assay sensitivity, we evaluated additional probe sets specific to novel high abundant targets for each pathogen (Pf EMP1 and Bm BMN) which dramatically improved detection of both parasites 4-30 fold. Utilizing these high copy probe sets against clinical blood samples has demonstrated 90-100% sensitivity. We also report on the development of PCR-based nucleic acid tests utilizing these same biomarkers for Pf and Bm parasites, detecting as few as 1 parasite/mL with a broad dynamic linear range allowing quantitation of parasite densities above 100 p/mL. Extensive data obtained during assay validation will be presented to highlight protocol-specific variables such as blood volume, reaction volume, and test sample volume that affect the sensitivity of both methods. For example, the performance of 18S target probes are dramatically improved when genomic DNA extracts are prepared from larger volumes of blood. Cumulatively, these data establish both the sandwich hybridization and PCR assays as sensitive pathogen detection platforms for diagnosis of chronically infected blood donors and provide useful insights on the development of general blood-based detection methods.

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COSTS AND AFFORDABILITY OF LAMP FOR MOLECULAR DIAGNOSIS OF MALARIA IN RESOURCE LIMITED, ENDEMIC SETTINGS

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Loopamp Malaria Pan/Pf Detection Kit (Eiken Chemical), based on the loop-mediated isothermal amplification of DNA (LAMP), is a simplified molecular method that offers PCR-equivalent diagnostic accuracy for the detection of low-density malaria parasitaemia and its speciation. Given its applicability in resource restricted field settings of malaria endemic areas, LAMP has considerable potential in shaping post-2015 strategies for global malaria control and elimination. We evaluated economic cost and affordability (in 2015 US$) of implementing LAMP as part of routine malaria control programs in two countries (Peru and Philippines) and compared it with that of smear microscopy and conventional PCR techniques. Per test costs were assessed based on a time and motion study that examined both direct and indirect resource inputs at various workload levels. Costs of LAMP implementation were calculated based on the laboratory workload and network data available in Peru. Per test cost of LAMP Pan or Pf followed an inverse exponential pattern against the workload levels with the cost stabilizing when at least 8 samples were processed per batch ($11-14). At highest workload levels (60 smears/88 PCR samples per batch), smear and PCR were $1.5 and $13 per test, but could cost as high as $7.5 (1 sample/batch) and $70 (4 PCR samples/batch). Implementing LAMP at 179 eligible laboratories (at least 1 to 5 samples per day) in Iquitos region in Peru, required $4.7 million per year for the first three years of implementation. Restricting implementation to 36 high workload laboratories (at least 12 samples per day), costs can be reduced to $2.1 million (contingency fund for malaria control in Peru for 2015 was $2.3 million). Though cost of LAMP can be competitive (vs. PCR), it will require considerable financial resources to achieve adequate coverage to meaningfully reduce time delays in malaria care. Likewise, cost-effectiveness or budget impact analysis evaluating various implementation strategies and their effects must be assessed to better guide policy decisions on future utility of LAMP for malaria control in high endemic, resource limited settings.

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FACTORS INFLUENCING THE USE OF MRDTS AMONG PRIVATE HEALTH PROVIDERS AND CONSUMERS AT THE KENYAN COAST

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In 2010, the Government of Kenya adopted the WHO test, treat and track policy which saw the introduction of malaria RDT testing in the public sector but limited support for the private sector. Fever seeking statistics indicate that 27% of people seek treatment in the private sector where availability of RDTs remains low at less than 20%. PS Kenya is implementing a fever case management project seeking to create a private sector market for increased access to and use of quality assured mRDTs. A qualitative study using In-Depth Interviews was conducted targeting private providers and patients in Kilifi, Mombasa and Kwale Counties. The study reached a total of 40 respondents i.e. 24 outlet personnel and 16 adult patients. Providers were trained and had 2 to 23 years' work experience. Patient interviews were conducted in private locations near but not affiliated with the health facilities. Interviews had no personal identifiers and were digitally recorded after obtaining interviewees' consent. All transcripts were analyzed and coded. Results showed that most providers reported using RDTs after training. They reported increased number of clients, ability to conduct out-of-office testing, reduction in workload /operation cost and patient preference and satisfaction. Concerns and challenges were 1) the lancets used were blunt and painful when pricking 2) inadequate buffer solution to conduct the test and 3) invalid results. Adoption of mRDTs depended on 1) trusted sources of the RDTs and type of brand 2) Purchase price for RDTs and costs to end-users. Overall, patients reported improved quality of care due to diagnosis before treatment and they liked seeing and interpreting results. In conclusion,Key factors that influence the use of the mRDT kit include ease of use of the kit, reliability of the test, reduction in provider work load and patient preference. Barriers include blunt lancets and inadequate amount of buffer- mRDT supply reliability, provider training and cost of mRDT to end user must also be addressed to ensure expansion of mRDT use in the private sector in emerging markets.

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EARLY POST-TREATMENT LEVEL OF HRP2 PREDICTS RECRUDESCENCE IN *PLASMODIUM FALCIPARUM* INFECTION

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Recrudescent infections following incomplete clearance of parasites are routinely reported for all classes of antimalarials. Traditionally, monitoring of response to antimalarial treatment has relied on serial blood microscopy. However, due to the limit of detection of microscopy, treatment failures are typically detected late, when the recrudescing infection has already reached a parasitemia high enough to cause symptoms. Stored longitudinal samples from 301 children enrolled in two therapeutic efficacy studies from 2013 and 2015 in Angola were analyzed, representing 243 participants with adequate clinical and parasitological response (ACPR), 39 with reinfection, and 19 with recrudescence. An ultra-sensitive bead-based Luminex immunoassay platform was used to quantify the concentration of the *P. falciparum* HRP2 protein in samples from follow up, ranging from 28 to 42 days. The HRP2 concentration on each day was normalized by the participant's HRP2 concentration on day 0, the first day of treatment. The concentration of HRP2 in participants with ACPR decayed exponentially after day 0, with a consistent decay rate of 6.5% (95% CI: 6.3-6.7) per day over the course of follow-up. Participants that ultimately suffered reinfections cleared HRP2 at an indistinguishable rate of 7.1% (6.4-7.8) per day prior to reinfection. In contrast, HRP2 concentration in participants that ultimately recrudesced behaved differently, increasing on average by 25% from day 0 to day 3, and was higher than in participants with ACPR or reinfections at all time points. The HRP2 concentration at day 3 was predictive of treatment outcome, with an area under the receiver operating characteristic curve of 0.86 (0.73-0.99). The remarkably consistent HRP2 decay rate in participants with ACPR and reinfections implies a common underlying biological clearance mechanism in the human body. Individuals that will ultimately fail treatment do not exhibit this same pattern of clearance, even in the absence of other indications of inadequate response to treatment. This raises the potential of using HRP2 concentration, particularly at day 3, to predict response to treatment.

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RELIABILITY OF RAPID DIAGNOSTIC TESTS TO ASSESS MALARIA TRENDS IN MADAGASCAR THROUGH A SENTINEL FEVER SURVEILLANCE NETWORK

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A sentinel fever surveillance network has been operational since 2007 in Madagascar. In all 34 sentinel sites, all febrile patients are tested with malaria rapid diagnostic tests (RDTs) for pan-LDH and pfHRP2, and data are monitored for diagnostically-confirmed malaria trends. Quality assurance of on-site RDT results are managed by the Institut Pasteur de Madagascar (IPM). Special attention is given to storage conditions and compliance with the manufacturer's instructions for RDTs. Results of RDTs stored at facilities are compared with results from the same RDT batch stored in ambient temperature <25°C and humidity <80% at IPM and also with microscopy. From January 2013 to December 2015, 33/34 sentinel fever surveillance sites were visited regularly throughout the country. There were no RDTs storage errors and no expired RDTs in stock at any sentinel sites. Most technicians (61/75, 81.3%) properly used RDTs in accordance with the manufacturer's instructions. The results of 1,638 febrile patients were used for quality assurance (3 invalid tests). Results of on-site RDTs and those stored at IPM were 99.8% concordant. Comparison with microscopy resulted in sensitivity of 92.5%, specificity of 97.1%, positive predictive value of 86.0%, and negative predictive value of 98.5% (n=1,635). These results indicate the reliability of malaria RDTs results from the fever sentinel sites. Thus, data collected at fever sentinel sites can be used by the National Malaria Control Program to better understand temporal and spatial trends in malaria transmission across Madagascar.

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COMPARISON BETWEEN A NOVEL COMMERCIAL ASSAY BASED ON LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) AND PCR-NESTED ASSAY FOR MOLECULAR DIAGNOSIS OF *PLASMODIUM* PARASITES

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Currently, approximately 2 billion people still live in areas at risk for malaria, with disease morbidity surpassing 200 million cases and about 500,000 deaths per year. Standard methods for malaria diagnosis include microscopic examination of blood films and rapid diagnostic tests (RDTs). Conventional and real-time PCR techniques are more reliable than either microscopy and RDTs in identifying *Plasmodium* species accurately, however, these molecular methods are technically challenging and resource intensive making them generally restricted to reference laboratories. Recently, Illumigene®, a panel of simplified assays based on LAMP technology (Loop Mediated Isothermal Amplification), has been developed for the diagnosis of major public health impact infections. In 2016, two assays have been set up also for malaria diagnosis (Illumigene® Malaria and Illumigene® Malaria PLUS, Meridian Bioscience Inc, Cincinnati, OH, USA), in collaboration with the US CDC and the University Cheikh Anta Diop (Dakar, Senegal). These assays are commercially available as CE IVD. In clinical studies, they have proven to be extremely sensitive, detecting up to ½ parasite genome per sample. The objective of this study was to evaluate both "Malaria" and "Malaria PLUS" assays for malaria diagnosis in 50 patients from retrospective Italian imported malaria cases, infected with *Plasmodium falciparum*, *P. vivax*, P. malariae and the two subspecies P.ovale wallikeri and *P. ovale* curtisi. These new Illumigene® LAMP-based assays were able to detect all *Plasmodium* species (including *P. ovale* subspecies) at genus level, and no discrepancies were found between these assays and our in-house nested-PCR. Product from the assay sample preparation could be also used for *Plasmodium* speciation by conventional PCR approach. The hands on for these new assays showed to be extremely simple, rapid (results in less than one hour) and thus suitable for all microbiology and hematology laboratories. All these characteristics open up new opportunities for exploiting new molecular tools for malaria diagnosis in endemic and non-endemic countries.

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COVERAGE AND IMPACT OF THE WHO-FIND MALARIA RAPID DIAGNOSTIC TESTS (RDT) EVALUATION PROGRAM: SHAPING THE GLOBAL MALARIA RDT MARKET

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Rapid diagnostic tests for malaria (RDTs) play a critical role in malaria case management, with quality being a key factor for good adherence to test results. The WHO-FIND Malaria RDT Evaluation Programme now functions since 2007, comprising a pre-purchase performance evaluation

(Product Testing, PT) and a pre-distribution quality control of lots (Lot Testing, LT). A small-scale survey in 2011 showed that the market-share of good performing RDTs has increased since then, however large-scale and recent data were not available. More generally, the current knowledge on the global malaria RDT market is still limited and mostly based on general trends, with little information about procurement practices. In order to better document the global RDT market, and to evaluate the current impact of the PT- and LT Programmes, a large-scale survey has been conducted, gathering RDT sales and procurement data from 2011 to 2014 from a total of 32 manufacturers, 12 procurers and 68 national malaria control programmes (NMCPs). The RDT sales results highlight a concentration of the market around 3 manufacturers (86% of 2014 sales), and a confirmed market shift towards RDTs complying with WHO procurement criteria (from 83% in 2011 to 93% in 2014). Procurement data showed that 74% of the NMCPs procure only 'complying' RDT products, however there is a frequent overlap of different products and even product types (e.g. Pf-only and Pf-pan) in the same year and country (60% and 46% of countries, respectively). Importantly, the proportion of 'non-complying' or 'not-evaluated' products was found to be higher in the private health care sector than in the public sector (32% vs. 5%), and even increasing over time (from 22% in 2011 to 39% in 2014). An estimated 70% of the RDTs market is covered by the LT programme. The opinion about the PT- and LT Programmes has been positive overall, and the quality of RDTs was rated as one of the most important procurement criteria. In summary, this survey provides in-depth information on RDT sales and procurement dynamics, including the largely unknown private sector, and demonstrates how the WHO-FIND Programme contributes to shaping the RDT market.

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ANTIPLASMODIAL ACTIVITIES OF CHLOROQUINE AND ARTEMISININ IN COMBINATION WITH VERBASCOSIDE, A PHENYLETHANOID GLYCOSIDE FROM *STACHYTARPHETA CAYENNENSIS*

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Verbascoside (VB), a phenylethanoid glycoside has been shown to possess strong antioxidant and immune-stimulant effects. The present study was designed to evaluate the antiplasmodial effect of VB purified from Stachytarpheta cayennensis leaves and its combination with artemether (Art) and chloroquine (CQ) in established *Plasmodium berghei* berghei infection in mice. VB was obtained by successive column separation of a methanol extract of *S. cayennensis* leaves; identity and purity of the compound were established by nuclear magnetic resonance spectroscopy (proton, carbon), high performance liquid chromatography and differential scanning calorimetric thermal analysis. In antiplasmodial tests, mice were inoculated on day 0 with chloroquine - sensitive *P. berghei* berghei NK65 infected blood. On day 3, mice were grouped (n=5) and respective groups treated for five days with VB, combinations of VB and CQ, combinations of VB and Art, CQ alone, or Art alone; at a dose range of 1-25 mg/kg of body weight. During treatment, thin films of tail vein blood of the mice were prepared daily and assessed for parasitaemia. After treatment, survival time was also monitored for all the experimental groups. The results showed that VB alone possessed significant ($P<0.001$) intrinsic antiplasmodial activity and exhibited synergism in combination with CQ, as increasing doses of VB significantly ($P<0.05$, 0.01, 0.001) boosted parasite clearance and prolonged survival time compared to CQ alone. Co-administered VB and Art produced significant ($P<0.001$), rapid and sustained parasite clearance within the first 4 days of treatment compared to Art alone, but survival time was highest in the group that received Art alone and was reduced with increasing doses of VB given in combination with Art. VB also produced a rapid onset action as observed in its ability to elicit rapid parasite clearance when administered alone or with the low doses of CQ or Art administered as monotherapy. Thus verbascoside warrants further investigation as possible combination agent in antimalarial regimens for uncomplicated malaria.

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FLAVONE DERIVATIVES: A PROMISING NEW CLASS OF DRUGS ACTIVE AGAINST MULTI-RESISTANT FALCIPARUM MALARIA PARASITES

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Plasmodium falciparum malaria is the deadliest parasitic disease with 438.000 deaths in 2013. The emergence and the increasing proportion of *P. falciparum* parasites resistant to artemisinin derivatives, the most potent antimalarials, is a major concern in Southeast Asia. Fast acting drugs, with unaltered activity versus the current multi-drug resistant strains are urgently needed to replace artemisinins. Previously, traditional remedies such as Cinchona bark or Artemisia aerial parts led to the discovery of the most potent antimalarials, bearing out that Nature is still an incredible source of original compounds. Following this approach, we are developing new synthetic antimalarial agents based on the structure of an active natural product. We isolated a biflavonoid from *Campnosperma panamense* (IC50 = 480 nM *in vitro* on *P. falciparum* K1 multi-resistant strain), and developed novel simplified synthetic analogs (MR series) with improved pharmacological and pharmacokinetic profiles. One of these compounds, MR70, is strongly effective on *P. falciparum* early blood stage in less than 6 hours. Moreover, MR70 and its analog MR87, exhibit a partial *in vivo* antimalarial activity, reducing parasitemia by 35% and 70% respectively on day 4 in a murine model (*P. berghei* ANKA, 100 mg/kg for 4 days). The investigations of structure-activity relationship are still ongoing to further improve these results. As MR70 acts specifically on early ring stage, which has been associated to artemisinin resistance, we have assessed the *in vitro* susceptibility of Cambodian artemisinin-resistant isolates to MR70 and found no cross-resistance between MR70 and artemisinins. These findings make flavone derivatives a promising new class of antimalarials. Further investigation is needed to optimize MR70 activity and assess its efficacy against strains resistant to partner drugs, usually combined with artemisinin derivatives, like piperaquine, mefloquine, lumefantrine and amodiaquine.

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DISCOVERY OF NEW HERB EXTRACTS TO TREAT MALARIA

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Malaria caused by *Plasmodium* spp. is found worldwide in tropical and subtropical areas. It is estimated that about 3.2 billion people - almost half of the world's population - are at risk of malaria. Especially, *P. falciparum* can cause severe malaria because it multiples rapidly in the blood, and can thus cause severe blood loss (anemia), cerebral malaria, and death. Currently, there are several drugs, chloroquine, atovaquone-proguanil, artemether-lumefantrine, mefloquine, etc. to treat malaria. However, an appearance of drug resistance malaria parasite is getting the greatest challenges facing malaria control today. To overcome this matter, a development of novel malaria drug is necessitated. Therefore, we evaluated antimalarial effect of 10 herb extracts used to treat febrile

patients in South Korea. Among the herb extracts, 50 µg/ml of two extracts obtained from mushrooms inhibited *P. falciparum* growth *in vitro* culture. In addition, lipoxygenase fraction of the extracts especially inhibited *P. falciparum* growth *in vitro* culture. Moreover, the lipoxygenase from the extracts inhibited chloroquine-resistant and atovaquone-resistant *P. falciparum*. Further study about the target molecule of the lipoxygenase fraction will be needed.

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MODULATION OF THE ANG/TIE2 AXIS REDUCES PATHOLOGICAL VASCULAR LEAK AND ACUTE LUNG INJURY IN A MURINE MODEL OF SEVERE MALARIA (SM)

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Malaria -induced acute lung injury (MA-ALI) carries a high fatality rate despite the use of potent antimalarial therapies and optimal supportive care. Therapies targeting the underlying pathophysiology of MA-ALI may be required to further improve clinical outcome. The angiopoietin-tyrosine kinase 2 (Ang-Tie2) signalling pathway is a key regulator of vascular integrity and recent evidence implicates Ang-Tie2 axis disruption in ALI development in ICU settings. We hypothesise that the Tie2 receptor ligand Ang-1, Tie2 and its activation are required to prevent MA-ALI, and that pharmacological approaches using pro-Ang-1, and Tie2 activation strategies will reduce or prevent MA-ALI. Respiratory distress (RD) occurs in ¬16% of SM and results in 39% of deaths , and is associated with Ang-Tie2 axis dysregulation in children and susceptibility in mice . Genetic models of Tie2+/- and Ang-1-/- mice will be infected with PbA-infected erythrocytes. We will determine MA-ALI markers of vascular leak (i.e. Evans Blue assay, IgM), histology and physiological dysfunction (i.e. O2 saturation) in the lungs and endothelial activation markers in lung tissue/plasma. We will administer pro-Ang-1, Tie2 treatment strategies alone or in combination at parasitaemia onset and compare MA-ALI markers with littermate controls. Pilot studies indicate that genetic disruption of Ang-1 results in decreased survival ($p=0.0091$), and increased pulmonary vascular leak as determined by Evans Blue dye assay ($p<0.05$) and pulmonary fluid accumulation ($p<0.05$). Tie2 activation using AKB9785, a phosphatase inhibitor that selectively inhibits VE-PTP, reduces IgM accumulation in the lung and vascular leak ($p<0.05$). We will report mechanistic studies of ALI in our genetic models and intervention studies using both pro-Ang-1 and other Tie2 activating strategies. Pilot results indicate that Ang-1 is necessary for the prevention of MA-ALI. Increased Tie2 activation shows evidence of reduced pathological leak and improved survival. These findings support targeting the Tie2 pathway with pro-Ang-1 and Tie2 strategies as adjunctive therapy for MA-ALI.

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PHARMACOKINETICS AND PROPHYLACTIC EFFICACY OF EMULSION OF DECOQUINATE FOLLOWING SINGLE INTRAMUSCULAR INJECTION IN MICE

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Prophylactic efficacy and pharmacokinetics (PK) were examined following single intramuscular (IM) depot formulation of decoquinate (DQ) emulsion injected into mice infected with *P. berghei* sporozoites. DQ nano-emulsion in 50% oily vehicle to retard drug release is suitable for long-term malaria prophylaxis. PK studies in normal animals and antimalarial efficacy in liver-stage malaria mice were conducted at various IM-DQ emulsion doses for 2, 3, or 4 weeks prior to infection with *Plasmodium berghei* sporozoites. The liver stage efficacy evaluation was monitored by using an *in vivo* imaging system (IVIS). Full causal prophylaxis was shown in mice with a single IM dose of large particle of nano-emulsion DQ (0.44 µm) at 120 mg/kg for 4 weeks and with small particle (0.18 µm) at 120 mg/kg lasted 2 weeks prior to inoculation. The 120 mg/kg IM emulsion dose was shown to be the minimal prophylactic dose required to provide full causal prophylaxis of malaria sufficient for a period of 2-4 weeks. A significant increase in the elimination half-life of the large particle DQ emulsion (632.15 hrs.) was achieved compared to that of the small particle DQ (494.47 hrs.). Similarly, the AUC of the large particle DQ nano-emulsion in plasma was observed to be 8,795 ng·h/ml, which is double the AUC observed for the small particle DQ emulsion (4,288 ng·h/ml) at the same single 120 mg/kg dose administered to both animal groups. Body clearance results indicated that the CL/F in the animals treated with nanoparticle DQ was 14.46 L/hr/kg, which is twice as fast as the clearance observed in animals treated with the microparticle DQ formulation (27.99 L/hr/kg). PK/PD evaluations have demonstrated the minimal inhibitory concentration (MIC) of DQ to provide full causal prophylaxis in mice infected with *P. berghei* sporozoites is 5.12 ng/mL. The large particle of DQ emulsion provided a longer and more constant DQ release in the plasma, which resulted in a 1.8 fold longer drug exposure time above MIC. The prophylactic effect of the large particle emulsion observed in mice was shown to be 2 times longer than the small particle of DQ nano-emulsion.

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ANTIMALARIAL POTENTIAL OF KOLAVIRON, A BIFLAVONOID FRACTION, FROM GARCINIA KOLA SEEDS, AGAINST *PLASMODIUM BERGHEI* INFECTION IN SWISS ALBINO MICE

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To investigate the antimalarial potential of kolaviron (KV), a biflavonoid fraction from Garcinia kola seeds, against *Plasmodium berghei* infection in Swiss albino mice. The study consists of seven groups of ten mice each. Groups I, II and III were normal mice that received corn oil, KV1 and chloroquine (CQ), respectively. Groups IV, V, VI and VII were infected mice that received corn oil, CQ, KV1 and KV2, respectively. CQ, KV1 and KV2 were given at 10-, 100- and 200-mg/kg daily, respectively for three consecutive days. Results indicate that administration of KV1 and KV2 significantly ($P<0.05$) suppressed *P. berghei*-infection in the mice by 85% and 90%, respectively while CQ produced 87% suppression relative to untreated infected group after the fifth day of treatment. Also, KV2 significantly ($P<0.05$) increased the mean survival time of the infected mice by 175%. The biflavonoid prevented a drastic reduction in PCV from day 4 of treatment, indicating its efficacy in ameliorating anaemia. Significant ($P<0.05$) oxidative stress assessed by the elevation of serum and hepatic malondialdehyde were observed in untreated *P. berghei*-infected mice. Specifically, serum and hepatic malondialdehyde levels increased by 93% and 78%, respectively in the untreated infected mice. Furthermore, antioxidant indices, viz; superoxide dismutase, catalase, glutathione-s-transferase, gluathione peroxidase and reduced gluathione decreased significantly ($P<0.05$) in the tissues of untreated *P. berghei*-infected mice. KV significantly ($P<0.05$) ameliorated the *P. berghei*-induced decrease in antioxidant status of the infected mice. Overall, the study shows that kolaviron at doses of 100 and 200 mg/kg elicits potent antimalarial activity in *P. berghei*-infected mice.

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EVALUATION OF ANTIMALARIAL ACTIVITY OF CYCLOPENTANONE AND CYCLOHEXANONE ANALOGUES OF CURCUMIN IN *PLASMODIUM BERGHEI* INFECTED MICE

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Malaria is one of the world's most common and serious tropical diseases. Resistance of *Plasmodium* to available antimalarial agents has necessitated the need to develop new antimalarial drugs which would be efficacious against drug resistant strains of malaria parasites. The *in vivo* antimalarial activity of cyclopentanone (CP) and cyclohexanone (CH) analogues of curcumin as potential antimalarial agents was evaluated using *Plasmodium berghei* mouse model. Female Swiss albino mice (n=55) were infected with standard inoculum (1x107) of chloroquine resistant strain of *Plasmodium berghei* (ANKA) intravenously. Infected animals were randomly distributed into eleven groups of five animals each. Once daily dose of chloroquine (10 mg/kg), twice daily graded doses (50, 100, 200 and 400 mg/kg) of each of the curcumin analogues and artemether/lumefantrine (4 mg/kg artemether) were administered to the infected animals while the control animals (infected but not treated) received polyethylene glycol (PEG, 100%), the vehicle for drug delivery twice daily for three days. All treatments were orally administered for three days starting from 24 hours post infection. Thin blood smears were prepared from tail snips of the mice daily between days 4 and 7 and subsequently on days 9, 12, 14 and 21, and parasite count was estimated by microscopic examination of Giemsa-stained thin smears. The result of this study showed that there was no significant difference in the antimalarial activity of both analogues of curcumin ($p>0.05$) on day four. However, the cyclopentanone analogue of curcumin at a dose of 200mg/kg demonstrated a recordable suppressive antimalarial activity (75% suppression). Cyclopentanone and cyclohexanone analogues of curcumin appeared to have weak suppressive antimalarial activity. Further research into the pharmacological properties of these analogues is recommended.

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TARGET-BASED DRUG DISCOVERY IN MALARIA: A NEW WAY TO IMPLEMENT AN OLD STRATEGY

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Compared to phenotypic screening, target-based drug discovery allows for a more directed medicinal chemistry optimization and a better prediction of the safety risks. Despite these advantages, the target-screening strategy has clearly lagged behind phenotypic screening approaches in malaria drug discovery. This is probably because of two hurdles: the often found disconnect between enzymatic and whole cell activity and the lack of robustly validated targets in *Plasmodium*. Recent advances in our capacity to identify validated drug targets in *Plasmodium* might provide a way to increase the success rate of target-based approaches in the near future. The Tres Cantos Open Lab Foundation (TCOLF) has recently pursued this approach in cases where a high level of genetic and small molecule validation has been achieved. Among others, current TCOLF-funded projects are working on identification of compounds that inhibit plasmodial N-myristoyl transferase (NMT) and cGMP dependent protein kinase (PKG). Projects are implemented in collaboration with GSK and academic experts, aiming to combine the in depth biology knowledge with the possibility to find the best starting points for medicinal chemistry optimization within GSK diverse small molecule compound libraries. We will present progress to date in the quest to identify target-based antimalarials.

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PRE-CLINICAL ASSESSMENT OF DRUG-DRUG INTERACTIONS BETWEEN PRIMAQUINE AND BLOOD STAGE ANTI-MALARIAL AGENTS

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The liver stage anti-malarial activity of primaquine and other 8-aminoqinoline molecules are directly dependent upon bio-activation through Cytochrome (CYP) 2D6 metabolism. Primaquine metabolism through the CYP 2D6 pathway makes the drug prone to CYP 2D6 mediated drug-drug interactions with concurrent medications that are CYP 2D6 substrates/inhibitors. Primaquine therapy is typically accompanied by administration of blood stage anti-malarial agents that clear the blood stages of the parasite and resolve the clinical symptoms of malaria. Concurrent primaquine-blood stage anti-malarial therapies have the potential to interact through CYP 2D6 and alter pharmacokinetics anti-malarial activity. We sought to investigate these interactions using an *in vitro* primaquine metabolism assay and recombinant CYP 2D6. In this study, commercially available blood stage anti-malarial agents were evaluated for the potential to interact with primaquine. The inhibitory potential of the blood stage anti-malarials for CYP 2D6-mediated primaquine metabolism were assessed *in vitro*. The blood stage anti-malarial agents tested displayed a range of inhibitory activities on CYP 2D6-mediated metabolism of primaquine *in vitro* (IC50 ranges 2-523 μM). Quinine was the most potent inhibitor (IC50 ~ 2.98 μM) of CYP 2D6 mediated primaquine metabolism. Artesunate and the artemisinin class were the least potent (IC50s > 200 μM). The *in vitro* inhibitory data was then used to predict *in vivo* interactions using the AUCin/AUC prediction method. The results indicate that primaquine interacts with several blood stage anti-malarial agents through CYP 2D6. The clinical implications of these interactions are currently unknown and warrant further investigation.

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THE COMBINED IMPACT OF TRANSMISSION-BLOCKING INTERVENTIONS AND PRE-ERYTHROCYTIC VACCINES FOR MALARIA ELIMINATION

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Transmission-blocking interventions (TBIs) aim to eliminate malaria by interrupting transmission of the parasite between hosts and mosquito vectors. Accurate methods to assess TBI efficacy are key to ensure that the best candidate TBI drugs or vaccines progress to clinical trials. This is particularly vital for novel population assays (PA) where efficacy is measured over successive transmission cycles. We present a method for estimating TBI efficacy from PA data by fitting a hierarchical Bayesian model to multiple life stages of the parasite. This enables both host-to-vector and vector-to-host transmission to be density-dependent processes whilst accounting for stochastic fluctuations driven by superinfection and small sample sizes. This improves the precision of intervention efficacy estimates and demonstrates that TBI impact is not sufficiently captured by changes in prevalence alone because TBIs also suppress parasite density in secondarily infected hosts. Partially effective TBIs require multiple

generations before substantial reductions in prevalence are observed whilst immediately suppressing parasite density. This has valuable implications for assessing the performance of TBI candidates in field and clinical trials.

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NOVEL ELQ-300 PRODRUGS FOR ENHANCED DELIVERY AND SINGLE-DOSE CURE OF MALARIA

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In the effort toward global malaria eradication, the discovery of a single-dose cure would facilitate ease of drug distribution and limit propagation of drug resistance, two issues that burden the current state of malaria treatment. One class of *Plasmodium* cytochrome *bc1*-inhibitory compounds, the endochin-like-quinolones (ELQs), are extremely effective against the parasite and have been shown to deliver single dose cures. Specifically, ELQ-300 is a pre-clinical drug candidate that is effective against all life cycle stages of *P. falciparum* and against blood stage parasites at low nanomolar concentrations. ELQ-300 is curative with three sequential low doses in treatment of patent malaria infection in murine models, but its low aqueous solubility and high degree of crystallinity prevent the higher bloodstream concentrations necessary to achieve a single dose cure. To accelerate the clinical development of ELQ-300 we have employed a prodrug approach, attaching a variety of bioreversible groups to ELQ-300 to increase bloodstream drug exposure. We have synthesized over 30 prodrugs of ELQ-300, many with a significantly lower degree of crystallinity than ELQ-300, aiding in solubility while retaining antiplasmodial activity. We will present a number of the most effective prodrugs that show excellent antiplasmodial activity *in vitro* and are curative with a single low dose *in vivo*. In addition to full antiplasmodial and pharmacokinetic profiles, we will present optimal formulations of spray-dried dispersions that improve aqueous solubility and oral bioavailability of these highly effective prodrugs.

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PLASMODIUM FALCIPARUM CYCLIC AMINE RESISTANCE LOCUS, PFCARL: A RESISTANCE MECHANISM FOR TWO DISTINCT COMPOUND CLASSES

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The emergence and spread of drug resistance to current antimalarial therapies remains a pressing concern, escalating the need for compounds that demonstrate novel modes of action and prevent the development of drug-resistance. As part of the Malaria Drug Target Identification Project efforts, we have adopted a chemogenomic approach to identify the targets of the most prominent compounds from chemically diverse libraries. Study compounds were selected based on availability, purity, potency in a multi-drug resistant isolate, and lack of known mechanism of action towards the mitochondrion or folate biosynthesis. Here we present studies of a drug-like compound from the Malaria Box, MMV007564, a novel antimalarial benzimidazolyl piperidine chemotype. To identify the genetic determinant of MMV007564 resistance, parasites were cultured in the presence of the compound to generate resistant lines. Whole genome sequencing revealed distinct mutations in the gene named *Plasmodium falciparum* cyclic amine resistance locus (*pfcarl*), encoding a conserved protein of unknown function. Mutations in *pfcarl* are strongly associated with resistance to a structurally unrelated class of compounds, the imidazolopiperazines, including KAF156, currently in clinical trials. Our data demonstrate that *pfcarl* mutations confer resistance to two distinct compound classes - benzimidazolyl piperidines and imidazolopiperazines. However, MMV007564 and the imidazolopiperazines – KAF156 and GNF179 – have different timing of action in the asexual blood stage and different potencies against the liver and sexual blood stages. Our results demonstrate that mutations in PfCARL mediate resistance to multiple chemical classes and might represent a common parasite drug-resistance pathway. Further characterization of PfCARL as a common drug-resistance pathway is underway.

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LEAD CANDIDATE SELECTION OF BROAD-SPECTRUM ANTIMALARIAL ACRIDONES

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We have previously reported the discovery of a novel antimalarial acridone chemotype that displays efficacy against sporozoite-induced *Plasmodium* infection in addition to efficacy against blood stage parasites. We have been successful in producing extremely potent new lead candidates with pico molar IC_{50} values against MDR resistant parasites, as well as full protection of liver stage infection at comparable dosage with primaquine. Details of the design, chemistry, structure-activity relationships (SAR), safety, metabolic studies, and mechanism of action will be presented.

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GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD) DEFICIENCY PHENOTYPE AND GENOTYPE DISTRIBUTION IN POINT-OF-CARE SETTINGS IN VHEMBE DISTRICT, LIMPOPO PROVINCE, SOUTH AFRICA

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South Africa is targeting malaria elimination by the year 2018. To facilitate transmission reduction, primaquine may be used as an additional chemotherapy to clear gametocytes, the transmissible stage of *Plasmodium*. Unfortunately, individuals with glucose-6-phosphate dehydrogenase deficiency (G6PDd) risk acute haemolytic anaemia if exposed to oxidant drugs, including primaquine particularly at high doses. A total of 248 subjects attending 6 primary health care facilities in Vhembe district, Limpopo province, South Africa were phenotyped for G6PDd using a commercial G6PDd test kit. Additionally G6PDd genotypes of the most common African forms G6PD A (A376G) and G6PD A- (G202A, A542T, G680T and T968C) were determined by polymerase chain reaction and restriction length fragment polymorphisms. There was 13.03% (33/248) G6PDd prevalence in Vhembe district as measured by the commercial testing kit. Of all males 18.2% (10/55) were G6PDd according to the commercial test, while females had a lower prevalence of 11.9% (23/193), Odds ratio 0.6088 (95% confidence interval 0.270 -1.371). The A376G/G202A genotype prevalence was 3.22% (6/248; 2.42% [N=6] male hemizygous and 0.80% [N=2] female homozygous). Heterozygous females were 12.90% (32/248) of participants. The A542T, G680T or

T968C variants were not detected in this locality. The sensitivity [95 % confidence interval (95 % CI)] and specificity of the commercial test kit to correctly identify G6PDd A- G202A deficiency were 90.38 % (95 % CI 85.54–94.03%) and 32.50% (95 % CI 18.57–49.13 %) respectively compared to genotyping. The agreement in test results was fair, Kappa value 0.246 (95% CI 0.090-0.413). There are low levels of G6PDd A376G/G202A mutations in the study locality. However, the poor specificity and agreement of the comercial G6PDd testing kit in comparison to the genotyping results call for development of additional robust field deployable point-of-care G6PDd test kits.

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SOCIETAL AND ENVIRONMENT CONTEXT OF PARENTS ACTING AS PREDICTORS OF SEVERE MALARIA IN CHILDREN UNDER 5 YEARS OF AGE ADMITTED IN KOUDOUGOU REGIONAL HOSPITAL, BURKINA FASO: A CROSS SECTIONAL STUDY

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Burkina Faso has a high incidence and death rate of severe malaria, especially for children under five years of age. Although the malaria elimination program is a cornerstone and high-priority public health project. Rating and understanding the various factors that contribute to the severity of malaria is useful in designing and conducting an effective strategy. In this study, factors associated with complicated malaria in Burkinabe children were investigated in semi urban city's hospital, Burkina Faso. Between Jun and September 2012, a cross-sectional study was used to test 510 children under 5 years of age (mean age: 32.23 months) admitted with suspected malaria. Each child was screened using two types of methods (test of diagnosis rapid and blood smear) to identify whether ill child had severe malaria focused on the criteria established by the World Health Organization. When a child was diagnosed with malaria, the relatives were interviewed by a trained nurse using a structured questionnaire to assess predicting determinants. A logistic regression using SPSS software version 17.0 was used to identify theses determinants of severe malaria and associated deaths. Of the 510 children having malaria, 203 (39.8%) had severe malaria. Most of the patients having severe malaria 86.2 % lived in rural areas. The main parental factors associated with severe malaria were delayed treatment [OR= 4.53, 95% CI = 1.76-11.65], low socioeconomic status [OR = 9.69, 95% CI (4.12-22.78)], a large household [OR = 2.28, 95% CI (1.50-3.47)], housewife [R = 16.39, 95% CI (2.18-122.9)], farmer [R = 19.39, 95% CI (4.61-81.44)]. The finding gathered from this study is one of the challenging and resources limited settings for emphasizing malaria elimination in children which remains a serious public health concern.Nevertheless understanding societal and environment contexts as possible predictors are still an important step towards the control of the disease. Improved health promotion, and encouragement to seek early care are urgently needed.

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IVERMAL: EFFICACY AND SAFETY OF HIGH-DOSE IVERMECTIN FOR REDUCING MALARIA TRANSMISSION - A DOSE FINDING STUDY

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Innovative approaches are needed to complement existing tools for malaria elimination. Ivermectin is a broad spectrum antiparasitic endectocide widely used for onchocerciasis and lymphatic filariasis control at doses of 150-200 mcg/kg. Ivermectin also has potent mosquitocidal properties, shortening the lifespan of mosquitoes that feed on individuals recently treated with ivermectin. However, the effect with the 150-200 mcg/kg dose is short-lived (6-11 days). Modelling suggests higher doses that prolong the mosquitocidal effects are needed for ivermectin to provide a significant contribution to malaria elimination. Ivermectin has a wide therapeutic margin, and previous studies have shown doses of 2,000 mcg/kg (i.e. 10x the FDA approved dose) are well tolerated and safe; the highest used for onchocerciasis is single-dose 800 mcg/kg. We are conducting a double-blind placebo-controlled, parallel-group, 3-arm, dose finding trial to determine the efficacy, tolerance and safety of 3-day courses of ivermectin 0, 300, 600 mcg/kg/day, when given in combination with standard 3-day course of dihydroartemisinin-piperaquine. We performed Monte Carlo simulations based on pharmacokinetic modelling to determine the dosing regimens to be tested. In our models, a dose of 600 mcg/kg/day for 3 days achieved similar median (5-95 percentiles) Cmax concentrations of ivermectin as single-dose 800 mcg/kg: 111 ng/mL (83-161) vs 108 (75-164), while increasing the median time above the MIC from 1.9 days (1.0-5.7) to 6.8 (3.8-13.4) days. The 300 mcg/kg dose was chosen at 50% to allow for a dose response. The clinical trial outcome of daily mosquito survival up to 28 days is assessed in laboratory-reared *Anopheles gambiae* s.s. populations fed on patients' blood taken at days 0, 2 (Cmax), 7 (primary outcome), 10, 14, 21, and 28 after the start of treatment. Safety outcomes include QT-prolongation and mydriasis. The sample size is 141 participants (47 per arm). Sub-studies include: (1) rich pharmacokinetics and (2) direct skin vs membrane feeding. The trial is ongoing in 6 facilities in western Kenya and will be completed in August 2016. Results will be presented.

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SPATIAL AND TEMPORAL VARIATIONS OF MALARIA RISK BETWEEN 2013 AND 2015 IN ZANZIBAR: A PRE-ELIMINATION SETTING

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The monitoring and evaluation of local transmission epidemiology to characterize malaria risk is essential for strategic planning of malaria elimination programs. Geographical Information System mapping techniques are a major set of tools for this approach to assess local time, spatial distribution and clustering of malaria cases in Zanzibar. In 2012,

the Malaria Case Notification system was developed to support individual case reporting. Each District Malaria Surveillance Officer (DMSO) was equipped with a tablet computer running a mobile application called Coconut Surveillance. Once a DMSO is alerted of a new case, he or she is guided through an active case response protocol by Coconut Surveillance. Additional case data are entered into the tablet at the facility and household. Each household member is tested and new cases are treated immediately. Coconut Surveillance uses the Geographical Positioning System (GPS) capability of the tablet to record the location of the household. Officials use near real-time maps and reports to quickly identify hot-spots and transmission patterns. Mapping of cases was done using Quantum-GISTM software version 2.12.0 and cluster analysis was performed using Bernoulli spatial scan statistic through the SaTScan® software. All 10 districts had malaria although incidence was lower in Pemba than Unguja. Unguja had an annual incidence increased from 6.3 per 1000 population in 2013 to 14.8 per 1000 population in 2015, of which 60% of cases in Unguja occurred between weeks 18 and 30 of each year. Among 9,352 cases identified during the surveillance, which were matched to 32,355 controls, we identified significant spatial clusters of malaria cases localized in Urban and West districts in Unguja, Wete and Micheweni districts in Pemba. Our results confirm the existence of a spatial heterogeneity and the seasonality of malaria transmission in Zanzibar, providing evidence for implementation of targeted interventions.

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IMPLICATION OF FLUCTUATING COMMODITY AVAILABILITY ON SUSTENANCE OF GAINS OF POPULATION BASED INTERVENTIONS

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Preventing malaria in pregnancy remains a daunting challenge in ensuring healthy mothers and newborns in malaria endemic communities. This study examines the effect of fluctuating antimalarial commodity support for the administration of intermittent preventive therapy for malaria in pregnancy (IPTp) using sulphadoxine pyrimethamine (SP), on the sustenance of gains of improved antenatal clinic visits and consequent reduction in the incidences of malaria among pregnant women accessing care in public health facilities across Enugu State, Nigeria. This study assessed clinical records of antenatal visits, administration of sulphadoxine pyrimethamine (SP), and confirmed cases of malaria among pregnant women during the high transmission period, across 255 health facilities. Trend analyses of percentage uptake of IPTp, were compared with and pattern of antenatal visits, and reported incidence of malaria over the study period. Findings: Using the first year as a baseline, observed 42% cumulative increase in antenatal visits in the second year appears to correspond significantly with the introduction of IPTp with an over 300% increase in uptake, and over 3% reduction in incidence of malaria in pregnancy (7.5% to 4.2%). With reduction in availability, IPTp uptake dropped in the third year to 5.6%, a proportionate reduction in antenatal visit of 6%, and malaria in pregnancy reduction was 1.6% (4.2% to 2.6%). As the availability dropped further in year 4 (-31.3%), the antenatal visits and malaria in pregnancy recorded relative marginal increases at 14.7% and 2.9% respectively. Further percentage reductions in antenatal visit at 22.4%, marginal increase in malaria in pregnancy incidence of 4.8% recorded in year 5 corresponds to the percentage reductions in IPTp uptake 23.6% occasioned by non-availability of commodities. In conclusion, it is imperative that interventions of which commodity supply is a critical component, has the tendency to impact positively on uptake and invariably produce and sustain desired results if emphasis is on ensuring unhindered commodity availability and improved access.

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HUMAN POLYMORPHISM ASSESSMENT AGAINST THE SAFETY AND EFFICACY USAGE OF PRIMAQUINE

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In all malaria-endemic countries outside sub-Saharan Africa, *Plasmodium vivax* infections are becoming the major challenge for control programs. The relapsing nature of *P. vivax* makes elimination difficult without radical treatment with 8-aminoquinoline drugs such as primaquine or tafenoquine against the long-lasting liver stage, hypnozoites. The safety and efficacy of 8-aminoquinoline drugs are however crucially dependent on each individual's glucose-6-phosphate dehydrogenase (G6PD) and cytochrome P450 2D6 (CYP2D6) genotype, respectively. Assessment of the impact of G6PD and CYP2D6 genotypes on the usage of 8-aminoquinoline drugs is hence important for designing optimal treatment schedules and drug-based public health inventions. A sensitive next-generation sequencing-based method to genetically characterise G6PD and CYP2D6 was established. This assay is suitable for large-scale screening strategy to evaluate the risk of primaquine therapy in different populations. Validation of assay performance will be conducted in a cohort of children (6 months to 12 years old) from Solomon Islands (2013-2014), which were tested for point-of-care RDT G6PD deficiency and treated with primaquine if clinical vivax malaria were observed. The ability to rapidly assess the genetic background for safe and efficient usage of primaquine in a given population has important implications for the planning of potential mass drug administration intervention programs. This study thus provides guidance for a better informed *P. vivax* elimination strategy.

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CHARACTERIZATION OF POPULATIONS CROSSING FORMAL AND INFORMAL BORDERS ON THE CAMBODIA-LAOS BORDER, INCLUDING IDENTIFICATION OF MALARIA INFECTION AND ARTEMISININ RESISTANCE INFECTION

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Recent evidence identified malaria prevalence among cross-borders to be as high as 11.5% in Cambodia-Laos border. As information is available for international border post which is not available at along the porous borders. The aim of this study is to estimate the potential contribution of crosser border to the spread of malaria and artemisinin resistance. A cross sectional study is being conducted at 7 informal and 1 international Cambodia-Laos border posts, in Stung Treng province. A total of 4500 individuals are expected to be tested between April 2015 and February 2016: sample collection started in September 2015. Participants are diagnosed malaria by using RDT and RT-PCR analysis. A structured questionnaire is capturing: demographic characteristics, history of travel, occupation. All crossers borders are eligible to participate and a written consent is required. Positive cases by RDT treat at the spot. Double entry questionnaire in EpiData 3.1 and PCR data is entered in Ms Excel database and then merged by unique identifiers in to STATA 12. A descriptive analysis of the primary data was conducted: demographic characteristics, RDT and RT-PCR results disaggregated by species. RT-PCR positive cases were also disaggregated and classified according to their fever status. A total of 291 samples were tested; 66.3% were male and the majority had

between 15-40 years old (63.2%); agricultural work was main occupation 68.7% but in Srei Champa Post, 20% of tested were security personnel. RT-PCR results: 16.2% of tested were deemed positive: informal border posts registered higher malaria prevalence than international border post (19.8% and 17.1% vs 11.4%). Among the positive, 87.2% were Cambodian and 12.8% Laos. RDT results indicated 2.7% were positive: international border post was also higher than informal posts (5.7% vs 0.9% and 2.3%). RT-PCR results: 65.1% of positive cases were asymptomatic: 16.3% were *P.f*, 69.8% *P.v*, and 13.9% *P.f/P.v.* Despite sample size limitations, differences in malaria prevalence among different types of border seem to exist.

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AN ASSESSMENT OF NATIONAL MALARIA SURVEILLANCE SYSTEMS FOR MALARIA ELIMINATION IN THE ASIA PACIFIC

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Heads of Government from Asia and the Pacific have committed to a malaria-free region by 2030. The Asia Pacific Leaders Malaria Alliance (APLMA) recognises that for malaria elimination to succeed, it is essential to have an accurate picture of malaria incidence over time and space. Having a closer look at each country's disease reporting system is a crucial component of this endeavour. It helps to recognise gaps in surveillance and develop evidence to support health system strengthening. This study describes the sources of malaria incidence data collected by national malaria control programmes (NMCPs) in 22 countries in the Asia Pacific targeting elimination. From April 2015, a short survey was sent to each NMCP. It collected country-specific information on existing sources of malaria incidence data, the system for collecting and collating these data and the role of the private sector in malaria treatment. Follow-up with key persons was done to ensure quality of survey responses, which were then stored in a secure database. Summary tables and thematic maps were generated to facilitate effective communication of findings to policymakers. Twenty-one countries completed the survey. Most of the malaria incidence data collected by NMCPs originate from government facilities, while many do not collect comprehensive malaria incidence data from mobile and migrant populations, the private sector or the military. All data from village health workers was included by 9/19 countries and some by 4/19. Other sources of data included police, plantations and other government ministries. Malaria is treated in private health facilities in 17/18 countries, while antimalarials are available in pharmacies in 15/18 and shops in 6/18. Countries should be supported to improve the completeness of malaria surveillance data collected from existing sources. In addition, a regional effort is warranted to include additional sources of malaria case data in the national surveillance database for each country, in particular from village health workers, mobile and migrant populations, the private sector, non-governmental organisations and the military.

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DECREASED ENDEMIC MALARIA IN SURINAME: MOVING TOWARDS ELIMINATION

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Suriname has moved from being the country with the highest annual parasite index in the Americas to one on the threshold of elimination. The progress toward elimination in the stable populations of Suriname between 2000 and 2015 is reviewed. Data were obtained from the Medical Mission and the Ministry of Health Malaria Program case-reporting systems, and analyzed with a focus on disease burden and differentiation of the disease geographically, by malaria species, age, ethnicity and trophocite and gametocyte infection rates. Between 2000 and 2015 there were 57.811 locally acquired cases of malaria in the stable populations of Suriname. A significant reduction in authochthonous malaria cases was observed from 2006 to 2015. The number of imported malaria cases is higher than the number of locally acquired cases since 2014, with a total of 10 imported cases vs 5 authochthonous cases in 2015. The overall decline in malaria case incidence can be attributed to active case detection in high risk areas, free distribution of impregnated bed nets in all transmission areas, public awareness campaigns and improved accessibility of diagnosis and treatment. The results from Suriname show how the local availability of good quality diagnosis and treatment are essential for the success of a malaria control program.

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RAPID REPORTING AND A SPATIAL DECISION SUPPORT SYSTEM TO STRENGTHEN MALARIA ELIMINATION INTERVENTIONS AND RESEARCH IN ZAMBEZI REGION, NAMIBIA

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As countries transition from control to elimination, surveillance systems must shift from periodic and aggregated case reporting to real-time reporting on individually geo-located cases. In Zambezi region Namibia, completed case forms often remained at health facilities (HFs), with delayed entry into databases that would permit finer scale analyses. To address these challenges a national rapid reporting system was piloted in western Zambezi (pop.~35381) and linked to a geographical reconnaissance (GR) of 8026 households conducted 2014-15. The GR mapped and collected baseline data on households and included a QR code affixed to each doorframe and resident's health passport. The rapid reporting system involves HF entry of minimum essential data on confirmed cases via tablet, sent by 3G network to a secure cloud database. A spatial decision support system (SDSS) was developed, incorporating a geographical information system (GIS)-based framework, a surveillance database, graphical maps, and expert knowledge. The SDSS includes GR data, retrospective 2012-14 incidence data from HF registries (including 3152 rapid diagnostic test-confirmed cases from 2013-15), and ongoing incidence data collected (2015-) by rapid reporting system. The SDSS enables users to plot cases to the household level within 24 hours of case detection, track progress by household during community-level investigations, and generate transmission risk maps. Challenges have included ensuring patients carry health passports and building capacity for nurses to complete the rapid report despite heavy workload. The Zambezi region SDSS has 3 roles: (i) to support Ministry of Health and Social Services (MoHSS) targeting of human and financial resources towards most at-risk areas; (ii) to support the implementation of a randomized controlled study exploring innovative case response strategies; and (iii) to coordinate the region's MoHSS and research activities. This represents one of the first reports of an SDSS being used to help a country move towards elimination, support research, and contribute to making that research more accessible and acceptable to local actors.

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DOCUMENTATION AND REACTIVE DETECTION OF MALARIA CASES IN A ZONE OF PRE-ELIMINATION FROM JULY TO SEPTEMBER 2015

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The ongoing decrease of the cases of malaria in Senegal has led the National Malaria Control Program (NMCP) to set a target of pre-elimination in the low transmission north and acceleration of control in the higher transmission south. In the north, malaria cases are investigated and reactive active case detection is conducted. Implementation started in 2011 in one northern district, and in 2015, the NMCP decided to progressively extend investigations in a northern region, adding three additional districts of the same region (St-Louis). Following extensive informational meetings and consultations with administrative, health and local authorities, health district personnel and community stakeholders were trained. Of the 580 cases diagnosed, 20% (115) were traveling through, and had no local address and 88% (508/580) were investigated. Of these, 59% of cases had traveled outside the district during in the previous 15 days. Among the 580 diagnosed cases, the 80% (465/580) who had addresses in the district were eligible for reactive active case detection within 7 days, with 82% (380) of these conducted within the correct time frame. Active case detection carried out in the household of the index case revealed 1.3% (57/4,466) test positivity, and in high risk members of five households around the index case, 0.6% (13/2,279) test positivity. Of the 70 additional cases detected through active case detection, 63 were in the household of the index case. The investigation of the cases of malaria in areas of pre elimination initiated by the NMCP and the responsibility of the district health management teams gave very satisfactory results. The NMCP plans to extend the strategy to other low transmission districts, integrating lessons learned to improve results and ensure success, namely involvement of all administrative and community actors, overseeing close and regular at the beginning and throughout the intervention, good coordination between the district teams and hospitals, and the need to consider focusing the investigation only at the household of the index case.

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COLLABORATIVE EFFORTS TO IMPROVE PREVENTION OF MALARIA IN PREGNANCY IN BURKINA FASO THROUGH USE OF IPTP-SP

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Malaria remains the first cause of consultation (47%), hospitalization (62%) and death (31%) in health facilities in Burkina Faso (2014 Statistical Yearbook). Pregnant women are among the most vulnerable to malaria. Intermittent preventive treatment in pregnancy (IPTp) is a priority intervention in the Burkina Faso 2011-2015 National Malaria Strategic Plan. In 2012, IPTp2 was low across the country at 53%. The President's Malaria Initiative (PMI) supported the National Malaria Control Program (NMCP) in implementing the national malaria control strategic plans. IPTp was promoted through 3 strategies: advocacy and policy updates, capacity building, and behavior change communication. Malaria prevention and management guidelines and job aids updated stressed IPTp in line with WHO recommendations. 185 trainers were trained who in turn organized one-day briefings for over 1,300 healthcare providers from 1081 health facilities (61.3% of health facilities nationally) on the revised guidelines, which were distributed along with job aids. Health information system tools now reflect new IPTp guidance, and 190 district and regional level data managers were trained in their use. 208 community health workers were trained in sensitization and community mobilization around early ANC attendance. Over 3000 radio and TV spots were aired on 28 stations on the importance of IPTp. In 21 project districts in 2013, IPTp2 and IPTp3 coverage rates based on ANC registration were 54% and 0%. Following the interventions, rates in these districts increased to 72% (IPTp2) and 23% (IPTp3) in 2014 compared to 63% and 8% in the other 42 districts. These efforts have resulted in improvements in IPTp service delivery and reporting, Based on successes, training and guideline dissemination continued in 2015 across the country so that all health facilities received copies of the new guidelines and 82% of districts received training.

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FINDING THE LAST FEW CASES: MOST EXPOSED INDIVIDUALS LESS LIKELY TO PARTICIPATE IN MALARIA SCREENING IN A PRE-ELIMINATION SETTING IN VIETNAM

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In a one-year cohort study comprising five malariometric screening surveys (n=456) in a remote, forested and recently endemic region of Central Vietnam, only 6 asymptomatic malaria infections (3 *Plasmodium falciparum* and 3 *P. vivax*) were identified, leading to speculation that local transmission may be fading out to elimination. However, people who spend overnights in forest fields, the most significant local risk factor for malaria, may be less likely to participate in screening due to regular absences from the villages where the surveys were conducted, leading to challenges in the identification of the remaining infection reservoir. Ancillary to the cohort study, an exploratory mixed methods study, comprising ethnographic fieldwork and a cross-sectional survey (n=160) was conducted to assess variation in malaria exposure-related behaviours and participation in screening. Full participation in all five screening surveys (40%) was not associated with spending overnights at forest fields (n=72), but individuals spending periods of one week to more than one month at their fields (n=16) were significantly less likely to participate in all screenings (12.5%). There was no difference in bed net availability by duration of stay, though 57% of bed nets at the fields had tears. In addition, people staying for longer durations at the fields were less likely to consistently sleep under a bed net, stayed in larger family groups, with fewer bed nets per person, and were twice as likely to have new unused LLINs stored in the village home, which suggests bed nets in use were older. Finally, those staying for longer durations at their fields had lower malaria knowledge. In conclusion, this study identified a sub-population with multiple malaria risk factors including extended stays in forest fields, lower effective bed net use, and lower malaria knowledge, but who are less likely to participate in screening surveys, which challenges elimination efforts. Mixed methods studies can improve the robustness of findings from small samples and highlight small populations in which the last few malaria cases are more likely to occur.

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IMPROVING IVERMECTIN´S EFFICACY AS A VECTOR CONTROL TOOL: REDUCING ITS METABOLISM AND EXCRETION TO PROLONG THE MOSQUITO-KILLING WINDOW

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The potential use of ivermectin mass drug administration to kill mosquitoes and reduce malaria transmission is gaining momentum. This endectocide has the potential to target mosquitoes feeding during the temporal and spatial gaps left by core vector control tools (insecticide treated nets and indoor residual spraying) and reduce residual malaria transmission, possibly easing the path to elimination. With a plasma half-life of 12-18 hours, however, either multiple daily doses or slow-release formulations are required to sustain effective mosquitocidal plasma concentrations. An additional alternative is the pharmacological inhibition of the metabolism and excretion of the drug. This strategy forms the basis of several drug combinations used effectively and safely in oncology and HIV treatment. Ivermectin is a substrate of both the CYP 450 (CYP) 3A4 and the P-glycoprotein (P-gp). We conducted an animal model experiment showing the effect of ketoconazole, a CYP3A4/P-gp inhibitor, in the oral pharmacokinetics (PK) of ivermectin. Six adult hybrid minipigs (37-60 kg) received a single oral dose of 800 mcg/kg of ivermectin. Blood samples for plasma-HPLC drug quantification were taken hourly for the first 8 hours and then at regular intervals for two weeks. The main PK parameters were calculated including the area under the curve (AUC) and the time above 6 ng/ml (a concentration known to kill 50% of biting *Anopheles gambiae*). The experiment was repeated after a wash-out period of one month, the animals were randomized to receive premedication with ketoconazole or nothing as a control before ivermectin. In animals pre-treated with the CYP3A4/P-gp inhibitor ketoconazole, we saw a statistically significant 6-fold increase in the time above mosquito-killing concentration and a significant increase in the AUC (1750 vs 464 ng/ml·hr). No sign of neurological toxicity was seen. Modifying the metabolism and excretion of ivermectin by means of CYP3A4/P-gp inhibitors appears to be an alternative strategy for prolonging its mosquito-killing effect and possibly increase its efficacy in reducing malaria transmission.

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CONTROL AND PRE-ELIMINATION OF MALARIA IN THE YUNNAN PROVINCE OF CHINA FROM 1983 TO 2013

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Yunnan faces an increasing risk of imported malaria cases from border countries, which Myanmar, Laos and Vietnam. With imported malaria thus becoming a major challenge in the context of malaria elimination in the province. The aim of this study is to understand the past and present malaria situation, and the challenges involved in its control, in Yunnan Province. A retrospective study of the past 30 years' of surveillance data and relevant sources on malaria in Yunnan was conducted. Data on malaria cases from 1983 to 2013 were collected from the China Information System for Disease Control and Prevention, as well as from case investigation reports.Results: From 1983 to 2013, a total of 375,602 malaria cases were reported in Yunnan Province; among these 739 resulted in death. Of the total number of malaria cases, 72.7% were infected with *Plasmodium vivax*, 21.2% with *P. falciparum*, 0.02% with *P. malariae*, 1.4% were mixed infection cases, and 4.7% were untyped cases. Out of the total number of reported cases, 207,956 were reported from the 25 border counties, accounting for 55.4% of the total malaria cases, and 44.6% (167,646) were reported from the mainland counties (the other 104 counties) of the province. The annual malaria incidence rate decreased from 64.8 per 100,000 in 1983 to 0.9 per 100, 000 in 2013. Among the 25 border counties, the malaria incidence rate decreased from 179.8 per 100,000 in 1983 to 4.5 per 100,000 in 2013, and the mainland counties malaria incidence rate decreased from 45.4 per 100,000 in 1983 to 0.3 per 100,000 in 2013. In 1983, malaria was prevalent in the northwest of Yunnan, Zhaotong city (northeast of Yunnan), and Yuanjiang-Honghe River Valley and border areas, while it was prevalent in the western and southern border areas of Yunnan in 2013. The population segment at high risk of contracting malaria consists of young male farmers and migrant workers. Conclusion: From 1983 to 2013, malaria control has been effective in Yunnan Province. Malaria has almost been eliminated in the mainland areas, and future control interventions should focus on the border areas.

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EFFECTIVENESS OF MALARIACONNECT FOR REAL-TIME CASE NOTIFICATION TO STRENGTHEN SURVEILLANCE IN SOUTH AFRICA

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South Africa is targeting malaria elimination by 2018. Strengthening the surveillance system is key to the country achieving its elimination goal. South Africa's historical paper-based notification system led to reporting delays before data was entered into the national malaria information system (MIS). To enable real-time case notification, South Africa developed MalariaConnect, an Unstructured Supplementary Service Data (USSD) tool for health care workers to notify malaria cases immediately from any mobile phone. MalariaConnect was deployed in November 2015 across 297 public health care facilities. These facilities are located in South Africa's five endemic districts (Ehlanzeni, Mopani, Umkhanyakude, Uthungulu, and Vhembe) and had previously reported at least 1 malaria case from 2012-2014. MalariaConnect notifications were compared against the paper-based data to assess consistency of reporting, and timeliness through a paired t-test. User acceptability has been assessed using an unstructured interviewer-administered questionnaire. From November 2015 through March 2016 there were 1234 cases reported through the paper-based system from facilities enrolled on the MalariaConnect project. On average, cases were notified through MalariaConnect within 1 day of patient diagnosis, compared to 5.1 days in the paper-based system (95% Confidence Interval 4.09-5.17: p 0.001). Of these, 70% (n=865/1234) were reported through MalariaConnect. Of the 108 healthcare workers that were interviewed and had used MalariaConnect, 96% (n=104/108) were willing to continue using the system. MalariaConnect has significantly improved timeliness of case reporting in South Africa and achieved high user acceptability. In 2016 South Africa will focus on increasing reporting rates through continued follow up visits and by strengthening systems for data-driven response.

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MALARIA ELIMINATION BY REACTIVE CASE DETECTION: CHALLENGES AND OPPORTUNITIES

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As several regions with historically substantial vectorial capacity approach eliminating malaria, there is a need to understand which features of a malaria surveillance and response system are most crucial for achieving local elimination and stemming resurgence. To investigate how local hotspots and spatial biases in surveillance quality interact to sustain local transmission, an individual-based model of malaria transmission, including vector life cycle dynamics and within-host immunity, was adapted to explicitly simulate transmission at the spatially-connected household level. A community of a few hundred households in Gwembe District, Southern Province, Zambia, where elimination operations are currently underway, was used as a model system. Vector bionomics, treatment-seeking, bednet usage, and mass drug campaign schedules and coverages were modeled according to household-level survey data from the area. Simulations predict that case management rate is the strongest determinant of success of reactive approaches, and high coverage and magnitude of response cannot completely compensate when a portion of symptomatic infections are left untreated. Rather than devoting resources to coordinating extensive reactive campaigns, we propose that a more effective strategy would be to improve access to treatment through strategic placement of village health workers and active community engagement on the importance of promptly treating cases.

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RWANDA TOWARDS MALARIA PRE-ELIMINATION: ACTIVE CASE INVESTIGATION IN A LOW ENDEMIC DISTRICT

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Rwanda has seen an increase in malaria cases recently with an increase from 514,173 cases in 2012 to 1,957,402 cases in 2015. This change can be attributed to an increase in temperature, rainfall, and resistance to insecticides. Despite this setback, Rwanda is aiming to reach the pre-elimination phase by 2018. In January 2015, 11 health facilities in Rubavu, a low endemic district, started implementing reactive active case detection after training 55 health care providers and 11 lab technicians on the topic. This strategy involves screening and treating individuals living in close proximity to passively detected cases, also known as index cases. Index cases can be used to identify population groups that are sources of infection. From January 2015 to December 2015, 16,434 cases of Malaria were detected and treated at 11 health facilities in Rubavu District. Among these cases, 2,917(17.8%) index cases were investigated and 4,943 individuals (between 1 and 2 contacts for each index case) living in proximity of index cases were tested using rapid diagnostic tests by health care providers. Of these, 508 (10.3%) tested positive for malaria and were treated according to national guidelines. This data shows that the number of investigated cases is still lower than the national guidelines of screening 5 individuals residing between 100 to 500 meters of every confirmed case. This low rate could be due to the increase of malaria cases in Rwanda which has placed a burden on health care providers and health facilities in areas like Rubavu which used to be low endemic malaria areas. Additionally, data gathered through supervision activities has indicated a need for additional training on screening investigations in order to adhere to national guidelines and conduct the investigations more efficiently. Active case investigation could be improved by training and involving more health care providers such as community health workers who could reduce the burden on health center staff. The additional support for case investigation activities and improved training can help to achieve higher coverage of individuals located near index cases.

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THE UTILITY OF MALARIA RAPID DIAGNOSTIC TESTS AS A TOOL IN ENHANCED SURVEILLANCE FOR MALARIA ELIMINATION IN VANUATU

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As part of efforts to eliminate malaria, Vanuatu has piloted the implementation of enhanced malaria surveillance and response strategies since 2011. This involves passive case detection (PCD) in health facilities, proactive case detection (Pro-ACD) and reactive case detection (Re-ACD) in communities using malaria rapid diagnostic tests (RDTs). While RDTs improve case management, their utility for detection of malaria infections in ACDs in this setting is unclear. We evaluated the utility of malaria RDTs as diagnostic tools in PCD, in five rounds of Pro-ACDs and five rounds of Re-ACDs conducted in Tafea and Torba Provinces, Vanuatu between 2011 and 2014. In PCD conducted in Tafea Province in 2013, a RDT-positive rate of 0.21% (2/939) and a PCR-positive rate of 0.44% (2/453) was observed in fever patients, demonstrating less than 1% of suspected malaria cases in this province were due to malaria. In Pro-ACDs conducted in Tafea and Torba Provinces, RDT-positive rates in 2013 and 2014 were 0.14% (3/2145) and 0% (0/2823), respectively, while the corresponding PCR-positive rates were 0.72% (9/1242) and 0.79% (9/1141). PCR identified villages in both provinces appearing to be transmission foci with a small number of low-density infections, mainly *P. falciparum* infections. In five rounds of Re-ACD, RDTs did not identify any additional infections while PCR detected only one among 173 subjects screened. These results demonstrate that both Tafea and Torba Provinces in Vanuatu has achieved very low malaria prevalence. In these low-transmission areas, conducting Pro-ACD and Re-ACDs using RDTs appears not cost-effective and may have limited impact on interrupting malaria transmission due to the small number of infections identified by RDTs and considerable operational resources invested. More sensitive, field deployable and affordable diagnostic tools will improve malaria surveillance in malaria elimination settings.

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BACK TO THE FUTURE: REVISITING THE ROLE OF CHLOROQUINE FOR MALARIA ELIMINATION IN MOZAMBIQUE

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Chloroquine (CQ) was used for many decades as the mainstay of antimalarial treatment, but was progressively abandoned due to increasing parasite resistance throughout the majority of malaria-endemic areas, particularly in Sub-Saharan Africa. Recent evidence from Malawi suggest that CQ sensitivity may be returning in places where discontinuation has reduced the drug pressure to the parasite populations. While this does not support the reintroduction of CQ as first line therapy, it suggests that, if proven sensitive in a given area, it could play a prophylactic role in malaria

elimination strategies when used in combination with other drugs or tools. Additionally, due to its high safety profile, CQ could also be considered an alternative for prophylaxis in first-term pregnancies and young infants.

A randomized, single-blind, placebo-controlled trial in asymptomatic Mozambican adults was conducted in the district of Manhiça, Southern Mozambique. Participants were followed up at days 0, 1, 2, 3, 7, 14, 21 and 28. The primary study endpoint was the rate of adequate and parasitological response (ACPR) to therapy on day 28 (PCR-corrected). Blood-slides and filter papers were collected at every study visit to measure parasite density and differentiate recrudescenses from new infections. A total of 52 and 27 participants were included in the CQ and Placebo group respectively. Mean parasite density at study entry was 517p/µL and there were 7 lost-to-follow-up participants in each arm. A PCR-corrected ACPR was 89% (95%CI 80%-98%) in the CQ arm and 36% (95%CI 17%-59%) among those in the placebo group (p< 0.001). In conclusion, this exploratory study suggests the return of CQ sensitivity in the South of Mozambique, implying its potential role as a prophylactic drug to be used in malaria elimination efforts such as mass drug administration, in combination with other effective anti-malarials. To further explore this question in Mozambique, additional studies will be performed on adults with clinical malaria and special populations.

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HETEROGENEITY OF *PLASMODIUM FALCIPARUM* MALARIA CASES IN MATABELELAND SOUTH: ANALYSIS OF CASES REPORTING TRAVEL HISTORY IN THE EARLY STAGES OF AN ELIMINATION PROGRAM

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As malaria prevalence decreases, imported malaria cases pose a challenge to malaria programs due to their potential to instigate local transmission. Travel history of RDT-confirmed cases, available through routine passive surveillance, was used to estimate potential for malaria importation in Zimbabwe's Matabeleland South province, where an elimination agenda was launched in 2013. Individual travel history records from 1,190 cases reported between September 2014 and June 2015 were analysed to quantify the number of cases travelling within Matabeleland South, to other parts of Zimbabwe and outside Zimbabwe. Reported travel locations of cases were geo-located. 185 (15.5%) of cases were reported to have travelled outside their home in the past 4 weeks, of which 99 (53.5%) were domestic travellers and 86 (46.5%) were international travellers. Beitbridge (63%) and Gwanda (21%) reported greatest proportion of cases that travelled. 44.3% of cases with travel outside Matabeleland South were identified in low transmission risk settings (<1 case per 1,000) and 20% were in high and medium transmission risk areas (>10 cases per 1,000) of the province. Environmental Health Practitioners classified 80 (6.7%) of all 1,190 confirmed cases as imported (transmission occurred outside Matabeleland South), 11 (0.9%) as intraported (transmission occurred outside case's home district but within Matabeleland South), and 744 (62.5%) as local (transmission occurred in home district of case). As locations reported in travel history data were described by coarse spatial resolutions, accurate classification was a challenge. Characteristics such as age, gender, and occupation will be explored to determine the demographic differences between travellers and non-travellers. Improvements in data collection, validation of travel history and its link to infections (including clustering of local cases) are needed for successful national and regional elimination of malaria.

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QUANTIFYING MALARIA-ATTRIBUTABLE FEVER IN AFRICA, AND THE DIFFERENCE IN TREATMENT-SEEKING RATES FOR MALARIA-COINCIDENT FEVERS AND NON-MALARIAL FEBRILE ILLNESS

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Malaria is typically assumed to be the causal parasite of febrile illness in Africa. While it is increasingly common for individuals to receive a diagnostic test for malaria before receiving treatment, it remains possible that the individual could have a co-infection with another disease that is in reality the underlying cause of their fever. Using paired observations of two-week fever history and *Plasmodium falciparum* malaria positivity from household survey datasets, we use model-based geostatistics to estimate prevalence of malaria-attributable fever, malaria-coincident fever, and non-malarial febrile illness across Africa. We show that febrile illness that is directly attributable to malaria accounts for a decreasing proportion of malaria-coincident febrile illness with increasing malaria prevalence, and that non-malarial febrile illness is widespread across the continent. Additionally, using household survey data on treatment-seeking rates for febrile illness, we show the difference in treatment-seeking rates between individuals with a malaria-coincident fever and non-malaria fevers. We show that if a substantial number of symptomatic malaria infections are not seeking care for their symptoms in low-transmission areas, then small-scale outbreaks may remain undetected, causing a problem for malaria elimination.

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SPATIAL DISTRIBUTION OF MALARIA CASES AND VECTORS IN A HYPO-ENDEMIC AREA OF WESTERN KENYA HIGHLANDS

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Malaria transmission in hypo endemic areas of Western Kenya highlands have been significantly reduced by extensive use of insecticide treated bed nets. However, residual transmission continues to maintain the disease in the community. Ecological studies suggest that there are foci of transmission. This study was undertaken to determine whether the distribution of malaria cases is random or clustered. Forty four microscopically confirmed index malaria cases from a local hospital were identified and homesteads of these patients geo-referenced. Household members in the index case population were screened for malaria. Houses located at a distance of 500 - 1000 m radius from the index case houses were identified and blood samples obtained for malaria diagnoses. This group served as the control. Mosquitoes were collected in the index case houses and control houses using pyrethrum spray catches. The number of malaria cases and vector density was compared between index case households and control population. In the index case household, the prevalence of malaria cases was 8 % compared to 4 % in the control houses. In the index case houses, vector density was 0.11 for *Anopheles gambiae* and 0.38 for *An. funestus*. In contrast, vector density in the control houses was 0.04 for *An. gambiae* and 0.27 for *An. funestus* .The malaria prevalence in the index case household was 2 fold greater than the control household. The indoor house density for *Anopheles gambiae* and *Anopheles funestus* were respectively 2.8 and 1.4 fold, greater in the

index houses compared to the control. Differences in the indoor density of the mosquitoes species were significant (P = 0.04). The data indicates higher transmission and malaria prevalence in the index case houses compared to the control, hence an indication of non-random vector and case distribution. Analysis of spatial distribution will be undertaken to show relationship between malaria cases and potential breeding habitats.

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SEVERE FLOODING AND MALARIA TRANSMISSION: IMPLICATIONS FOR DISEASE CONTROL IN AN ERA OF GLOBAL CLIMATE CHANGE

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There are several mechanisms by which global climate change may impact malaria transmission, including those that relate to changes in the frequency of extreme weather events including heat, drought, and floods. We sought to assess how the increased frequency of extreme precipitation events associated with global climate change will impact malaria transmission in highland areas of East Africa. We used a differences-in-differences, quasi-experimental design to examine spatial variability in the incidence rate of laboratory-confirmed malaria cases and malaria-related hospitalizations comparing villages at (1) high vs. low elevations, (2) with and without rivers, and (3) upstream vs. downstream before and after severe flooding that occurred May 1, 2013 in the Kasese District of Western Uganda. Findings: During the study period 7,596 diagnostic test were performed and 1,285 patients were admitted with a diagnosis of malaria. We observed that extreme flooding resulted in an increase of approximately 30% in the risk of an individual having a positive malaria diagnostic test in the post-flood period in villages bordering a flood-affected river compared with villages further from a river with a larger relative impact on upstream vs. downstream villages (adjusted RR 1.91 vs. 1.33). In conclusion, extreme precipitation such as the flooding described here may pose significant challenges to malaria control and elimination programs, and will demand timely and sustained responses to prevent and mitigate deleterious impacts on human health.

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MALARIA IN THE FIRST TRIMESTER OF PREGNANCY: INCIDENCE AND ASSOCIATED RISK FACTORS IN BENIN, SUB-SAHARAN AFRICA

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In Africa, preventive drug strategies against malaria in pregnancy are recommended from the 2nd trimester and bed nets are rarely used in early pregnancy. Therefore, women remain insufficiently, or not, protected during the first trimester, when malaria may be particularly deleterious for the mother and the child. The incidence of and risk factors associated with malaria in the first trimester have been poorly explored so far. A subsample of 200 pregnant women recruited before conception, were followed up monthly until delivery. Malaria was detected during the 1st, 2nd and 3rd month of pregnancy. A multivariate mixed model was used to assess factors associated with malaria during the first trimester. The cumulative incidence of malaria during the first trimester of pregnancy was 17.8% (11.2% during the first month). Early gestational age (≤ 6 weeks' gestation) (aOR: 2.69 [1.35-5.37]) and living in a lakeside area (aOR: 0.17 [0.04-0.85]) were the main factors significantly associated with malaria in the first trimester. In conclusion, malaria was highly incident during the first trimester of pregnancy, particularly during the first month and in women living far from the lake. The consequences of these infections for the mother and her child need to be assessed.

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FIRST PILOT PROJECT FOR ACTIVE SURVEILLANCE OF ASYMPTOMATIC MALARIA CASES IN HISTORICALLY ENDEMIC REGIONS IN PARAGUAY BY TWO METHODS MICROSCOPY AND MULTIPLEX SEMINESTED PCR

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In 2014, Paraguay was placed among the 16 countries that reported zero indigenous cases of Malaria. The implementation of active surveillance to detect potential asymptomatic cases in historically endemic regions in Paraguay is needed considering the indigenous parasite is *Plasmodium vivax* that contrary to *P. falciparum*, can remain latent as hypnozoites in the host. In the present study we applied the microscopy and the molecular diagnosis to search for sub-patent parasitemias in asymptomatic cases. Adults from a total of 15 localities from three departments, Alto Paraná, Caaguazú and Canindeyú were selected, based on the records of the SENEPA considered as the last localities that reported at least one case in the period of 2007-2011. The sample size obtained for 95% confidence was 361 but 332 samples were collected and analyzed up to now and informed consent of each of them was obtained. Thick smears were analyzed by microscopy. DNA samples were extracted from blood drop dried on filter paper and analyzed by the Seminested Multiplex PCR using the primers that amplify the 18 S rDNA for the four species that cause Malaria (*P. falciparum*, *P. ovale*, *P. malariae* and *P. vivax*). Human 18S rDNA was amplified as internal control. Sensitive essays allowed to detect until 0,01 ng of genomic *P. falciparum* DNA. From the 332 samples, 11.4% were from the department of Canindeyú, 30.1% from Alto Parana and 58,4% from Caaguazú. The distribution per locality was: 11,4% from Pira Vera, 10.5% from Maracamoa, 7.8% from Nueva Esperanza, 11.7% from Mision Verbo Divino, 2.4% from San Juan, 1.8% from Mbarigui Indígena, 17.2% from Mil Palo, 0.9% from Nueva Brasilia, 4.2% from Nueva Esperanza, 11.1% from Nueva Toledo, 1.8 from Ñu Jhovy, 3.6% from Pindoí, 2.4% from Santa Clara, 7.5% from Santa Teresa and 5.4 from Yby Moroti. Fifty seven percent were female and 43% were male. We could not detect any sub-patent parasitemia that can reveal the presence of asymptomatic cases. The results obtained in this study are very promising for our country, at this stage where all the efforts are done towards the eradication of the Malaria.

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MALARIA IN AN INTERNALLY DISPLACED PERSONS CAMP IN THE DEMOCRATIC REPUBLIC OF CONGO

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Malaria remains a leading cause of death in children under 5. Malaria prevention and treatment efforts in the Democratic Republic of the Congo (DRC) are hindered by armed conflict, which has resulted in the displacement of approximately 2.7 million individuals. This study aimed to describe malaria cases treated at a health centre serving an internally displaced persons (IDP) camp in DRC. The study took place in Luchebere IDP camp in the DRC which housed 2,580 individuals (318 children <5) at the time of the study in 2014. The camp consisted of two waves of IDPs: (1) ~1,400 individuals from Masisi and Walikale in Sept 2013; and (2) ~1,500 individuals from Northwest Masisi in Jan-Feb 2014. Febrile patients

presenting to the only health clinic in the area, which provides free medical services to IDPs, were tested for malaria using a rapid diagnostic test (Paracheck®). Demographic and clinical data were abstracted from clinic records. Uncomplicated malaria cases were treated with artemisinin-based combination therapy, according to WHO recommendations, and severe malaria with intravenous quinine. Between January and July 2014, 751 patients presenting to the clinic with fever were tested for malaria. 323/751 (43%) tested positive, including 169/279 (61%) children <5. The incidence of malaria requiring treatment in the IDP camp was estimated to be at least 210 per 1,000 at risk per year overall and 910 per 1,000 at risk per year in children under 5 (>3-fold higher than the WHO Africa region). Of the participants with malaria, 292/323 (91%) had uncomplicated disease and 28/323 (8.8%) had severe malaria. There were 4 deaths, 2 in children <5. 452/751 (60%) of patients had a bednet in their shelter and of these, 259/452 (59%) reported using the bednet. Malaria accounted for a higher proportion of febrile illness among patients from the second wave of IDPs who had lived in the camp for <6 months (72% vs 12%, p<0.0001), and among those who reported that they did not use a bednet (62% vs 8.9%, p<0.0001). The findings suggest that control measures targeting this high risk population may reduce the burden of malaria, particularly children under 5 and recent arrivals to the IDP camp.

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PREVALENCE OF SUBMICROSCOPIC MALARIA IN PREGNANCY IN COLOMBIA

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For countries progressing towards malaria elimination, highly sensitive diagnostic methods are required to target reservoirs of transmission. Currently, microscopy is the gold standard for malaria diagnosis however, more sensitive tests are needed to identify low-density submicroscopic infections. Pregnant women are an important risk group as they may harbour submicroscopic infections which could impact clinical outcomes for the mother and fetus. Here, a reverse transcription real-time PCR (RT-qPCR) was developed for detection of the 18S rRNA gene for *Plasmodium falciparum* and *P. vivax*. The assay was validated with 42 blood samples and demonstrated a specificity and sensitivity of 100% when compared to qPCR. To investigate the prevalence of submicroscopic malaria in pregnancy, a cohort of 200 women from Colombia were recruited and followed longitudinally via antenatal visits until delivery. Of the 38 women followed up so far, 19 were positive for submicroscopic malaria during pregnancy. 228 samples were screened by RT-qPCR, of which 27 samples tested positive. 22%, 59%, and 19% of the infections were caused by *P. falciparum*, *P. vivax*, or mixed, respectively. Additionally, samples from asymptomatic subjects and from febrile patients were collected from Colombia and tested via microscopy and RT-qPCR to investigate the frequency of submicroscopic malaria outside of pregnancy. Of the 84 asymptomatic samples screened by RT-qPCR, 6 and 10 were positive for *P. falciparum* and *P. vivax*, respectively. Further, of the 322 febrile samples screened by RT-qPCR, 36 and 66 were positive for *P. falciparum* and *P. vivax*, respectively. These results reveal a high frequency of submicroscopic malaria in this region. We report that 50% of pregnant women followed up in this study were positive for a submicroscopic infection at some point during pregnancy. 15% of the asymptomatic population and 27% of the febrile population were also positive for submicroscopic malaria. We propose that the use of more sensitive tests and active surveillance during pregnancy will become a necessity for the end goal of malaria elimination in this part of Latin America.

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CORRELATION BETWEEN MALARIA INFECTION DURING PREGNANCY AND ADVERSE BIRTH OUTCOMES IN VARIED MALARIA TRANSMISSION SETTINGS IN UGANDA AND BURKINA FASO RESPECTIVELY

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Preterm delivery and low birth weight are the leading causes of neonatal mortality and morbidity in children below the age of five years. The mechanisms under which malaria leads to low birth weight, still birth and preterm labor are poorly understood. Using data from a prospective cohort in which; 990 pregnant women were enrolled, 853 (85.2%) followed through delivery and resulting in 838 live births, we assessed the association between malaria infection during pregnancy and adverse birth outcome that included low birth weight, still birth, abortions and preterm delivery in two African clinical settings (Uganda, hyperendemic, and Burkina Faso, seasonal transmission). Women were categorised according to their malaria infection history during the study: Baseline, high parasite group and low parasite group. A total of 338 (40.8%) were in the baseline group, 310 (37.4%) were in the high-level infection group, 120 (14.5%) were in the low-level infection group, and 60 (7.2%) had occult or early infections. Overall there were 230 adverse birth outcomes across the two study sites; this represented 27.0% of all births: Uganda (83) and Burkina Faso (147). Premature births represented 74% of adverse outcomes. The proportion of preterm births was highest in low parasite group (0.215) in Burkina Faso and (0.296) in Uganda. Infection group was not found to be a significant predictor of an adverse outcome (P>0.05) using logistic regression. Although proportion of infants weighing <2.5kg was highest in the occult infection group (0.169), the proportion of infants with low birth weight was not significantly associated with infection group (P=0.257) even after adjusting for potential confounders (age, gravidity, country and weeks of amenorrhea at delivery); infection group did not significantly influence the odds of having a low birth weight infant (P=0.603). In the cohort of women enrolled in this study, malaria infection during pregnancy was not statistically associated with adverse birth outcomes.

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MAPPING CLIMATIC, GEOGRAPHIC AND SOCIO-ECONOMIC DETERMINANTS OF MALARIA IN MALAWI FOR MALARIA RISK ASSESSMENT

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The 2016-2030 WHO global technical strategy for malaria aims to reduce malaria case incidence by 90% in 2030. Achieving this will not only depend on new interventions but require optimal use of novel technologies and methods. More accurate profiling of geographical variation in malaria over time, aimed at identifying transmission hotspots, will enable more targeted control. Modelling interactions between various potential drivers and their effect on malaria risk can also lead to a better understanding of transmission dynamics. Modern spatio-temporal statistical models and Bayesian predictive inference are particularly well suited to mapping health outcomes in low resource settings for (at least) three reasons. Firstly, they enable more precise prediction in data-sparse

regions by exploiting spatio-temporal dependence in the health outcome of interest in addition to associations with spatio-temporally dense environmental covariates. Secondly, they can simultaneously capture spatial variation at large and small scales. Finally, they deliver honest assessments of predictive uncertainty. We use spatio-temporal statistical models to investigate the contribution of climatic, environmental and socio-economic factors to district-level variation in malaria risk in Malawi. Outcome data (malaria cases) are taken from an age stratified health management information system data covering all 28 districts in Malawi between July 2004 and December 2015 while socio-economic data are obtained from national surveys. Remotely sensed climate data averaged over the districts are used to capture the impact of climatic variations on transmission. We first assessed covariate effects in a non-spatial model to identify the most important significant drivers of malaria, which we then used to build a spatio-temporal model and generate Bayesian predictive maps of spatial variation in disease risk. These predictive maps serve two purposes: to highlight areas of unusually high and low risk that could inform sub-district surveillance and control strategies; and to target augmented sampling designs on areas where current predictions are imprecise.

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RURAL-URBAN DIFFERENCES IN THE UTILIZATION OF MALARIA PREVENTIVE AND TREATMENT SERVICES BY WOMEN OF REPRODUCTIVE AGE GROUP IN NIGERIA

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Nigeria witnessed a dramatic improvement in the provision of universal access to malaria prevention, diagnosis and treatment tools. The objective of this analysis is to describe rural urban differences in the utilization of malaria preventive and treatment services in Nigeria by women of reproductive age group to inform malaria control programming and planning. A cross sectional survey involving 6,344 women aged 15-49 years selected through multi staged cluster sampling procedure. Descriptive analysis was done to obtain weighted prevalence and proportions of women background characteristics and selected outcome variables. Univariate and multivariate logistic regression analyses were then conducted to obtain crude and adjusted odds ratios for the hypothesized association. Statistical testing was done using the adjusted Wald test. An estimated 30% of the women reported the use of net the night before the survey. 30% of them claimed their children had experienced fever in the preceding 2 weeks before the survey but only 14% had a malaria confirmatory test for their children. An estimated 65% percent of the women who sought treatment for their children used the private sector. Rural women have about twice the odds of using a net than their urban counterparts (2.12(1.47; 3.06), p<0.001). The odds of using nets decrease with any form of formal education, with increasing wealth index and among older women. Further multivariate analysis showed that younger, rural women are more likely to use net OR (1.77(1.12; 2.80) p=0.013) while the inverse relationship between level of education and net use persisted. There is no evidence of an association between place of residence and malaria diagnostic testing. Utilization of net is generally low with more tendencies to use net among rural, un-educated and lower socio economic status. Treatment of malaria based on clinical suspicion is widely prevalent and majority of the women patronize the private sector. Recommendations were made for public health strategies to encourage use of nets; improve infrastructures for malaria diagnosis and strengthening of the health system.

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DESIGN OF A CLUSTER SURVEY STUDY TO DESCRIBE THE EPIDEMIOLOGY OF MALARIA GAMETOCYTE CARRIAGE AND TRANSMISSION DYNAMICS IN A HOLOENDEMIC TRANSMISSION SETTING IN KISUMU COUNTY, WESTERN KENYA

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To better target malaria control interventions, more information is needed about the human, mosquito and environmental factors that affect who is carrying gametocytes and who among these carriers is transmitting malaria. While human and parasite factors, including the intensity and duration of gametocytemia, affect infectiousness at the individual level, entomological factors such as mosquito exposure dictate the transmission potential of each individual and the infectious reservoir as a whole. Most malaria transmission studies have not measured these factors simultaneously and longitudinally. Here, we describe the design of a pilot cluster design survey study that is simultaneously and longitudinally assessing human, parasite and entomologic factors that influence malaria transmission in Kisumu, Kenya. The study area is a 369km2 region located on the northeastern shores of Lake Victoria that has been mapped using Global Positioning System (GPS) technology and divided into clusters that measure 1km by 1km. Thirty clusters were randomly selected, ensuring at least a 1 km buffer zone around a selected cluster and that each selected cluster had at least 4 households with each household having at least 1 person in all of the following age categories: above 25 years, below 5 years and between 6 and 25 years. Field workers with GPS devices were sent to the homesteads to obtain consent from the heads of the households. The clusters cover 18% of the total study area. Each cluster is visited weekly and each homestead monthly to collect resting mosquitoes as well as epidemiological data and blood samples from the study participants. Testing for the presence of asexual and sexual stages by microscopy and molecular methods is performed on the samples collected from the study participants. Female mosquitoes are dissected and tested for the presence of *Plasmodium* sp. An analysis of blood meal is done for fed female mosquitoes. Individuals identified to harbor gametocytes undergo mosquito feeding assays; a subset of adults undergo both direct and membrane feeding assays for direct assay comparison. Preliminary findings will be presented.

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INCREASE IN MALARIA AWARENESS AND REDUCTION IN MALARIA PREVALENCE IN ENDEMIC DISTRICTS OF BANGLADESH: EVIDENCE FROM FOLLOW UP MALARIA PREVALENCE SURVEY 2013

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Malaria is endemic in 13 districts of Bangladesh. A baseline malaria prevalence survey across the endemic districts of Bangladesh in 2007 was conducted by our group, when the point prevalence was reported around 39.7 per 1,000 population. Followed by the two rounds of Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) funded intervention by

National Malaria Control Programme and BRAC led NGO consortium we had conducted a follow up survey during August to November 2013 in 70 upazilas (sub districts) of 13 malaria endemic districts of Bangladesh to measure the reduction following GFATM interventions.. We used a multi-stage cluster sampling technique to collect 9750 blood samples from same number of households. We used "FalciVax" rapid diagnostic tests (RDT) to diagnose malaria from blood from randomly selected individual from a household and the test result was recorded. The same RDT was used during the baseline survey. As in the baseline survey, the household head or available eldest person was interviewed using a pre-coded structured questionnaire to collect data on the knowledge and awareness to malaria of the household. Based on weighted calculation, overall malaria prevalence was found 1.4 per 1,000 population. The proportion of *Plasmodium falciparum* mono infection was 78% while *P. vivax* and mixed infection of these two species were 11% in both cases which was 90.2% for *P. falciparum* mono infection 5.3% and 4.5% for *P. vivax* and mixed infection during 2007. Bandarban was the highest malaria prevalent district (6.7 per 1,000 population) in the follow up survey. Knowledge on malaria sign and symptoms and mode of transmission were found better in follow up survey (97.3%) than the baseline survey (34.0%). Use of insecticide treated bed nets for prevention of malaria was found high (90.2%) at the respondents level during follow up survey. Overall, people in Chittagong hill tracts areas had slightly better knowledge than those in other areas. In a nutshell, a reduced point prevalence of malaria and increased level of knowledge were reported in the follow up malaria prevalence survey in Bangladesh.

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SEASONAL CHANGES IN THE ANTIBODY RESPONSES AGAINST *PLASMODIUM FALCIPARUM* ANTIGENS ON ISLANDS IN LAKE VICTORIA, KENYA

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Anti-malaria antibody responses can help characterize heterogeneous in malaria transmission. In the present study antibody responses to *Plasmodium falciparum* AMA-1, MSP-119 and CSP were measured to assess the transmission intensity in meso-endemic settings in Lake Victoria. Two cross-sectional surveys were conducted in dry and wet seasons in 2012 comprised of five settings: Ungoye (mainland), Mfangano (large island) and small islands (Takawiri, Kibuogi, Ngodhe). Individuals provide a finger-blood sample to assess malaria infection by rapid diagnostic test (RDT). Exposure to malaria antibodies were detected by ELISA using eluted dried blood form filter paper. Of 5044 participants, RDT tests were done in 4852 (96.2%) and 4112 (81.5%) were tested for serology. The overall seroprevalence was 64.0% for AMA-1, 39.5% for MSP-119, and 12.9% for CSP. Within settings, seroprevalences for merozoite antigens were higher in Ungoye and Mfangano than in small islands, showed different patterns between seasons and consistently high in the wet season for CSP ($p<0.01$). The overall seroprevalence and antibody titers generally increase with age group ($p<0.001$). Seroconversion rates (SCR) demonstrated different patterns between seasons where AMA-1 seroconversion rates constantly high and similar in Ungoye, decreased in Mfangano and Takawiri but increased in Kibuogi and Ngodhe from dry to wet seasons. Increasing age was strongly associated with increased odd of seropositivity in all settings. We observed heterogeneity of parasite prevalence and serological indices acroos study sites and temporal changes in the force of infection islands in Lake Victoria. These data suggest that AMA-1 sero-epidemiological analysis may have a role in assessing short-term changes in exposure especially in high or seasonal transmission settings and appeared to best reflect transmission intensity.

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IMPACT OF ANTIMALARIAL INTERVENTIONS ON MALARIA MORBIDLY AND MORTALITY IN HOSPITALS FROM 2001-2015, SENEGAL

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The Senegal National Malaria Control Program and partners accelerated malaria control in the last decade, with nationwide roll-out of artemisinin-based combination therapy (ACT) starting in 2006, and rapid diagnostic tests (RDTs) starting in October 2007. Since 2003, insecticide-treated nets (ITNs) have been distributed through health facilities to pregnant women and children under five years, and since 2008, mass campaigns of ITN distribution have targeted children under five years, with universal coverage starting in 2010, and 2014. Since 2007, indoor residual spraying has been implemented in seven districts. We assessed the trends of malaria cases, hospitalizations and deaths at 35 hospitals and in all 76 districts during the period of the scale-up of malaria control interventions. Data collected from all hospitals and districts from 2001-2015 were used to assess the impact of accelerated malaria control. Numbers of outpatient and inpatient cases and deaths were compared between the 2001-2007 period and the accelerated - intervention period of 2008-2015. From 2001 to 2007, the proportion of suspect cases confirmed increased slightly from 2.2% to 4.0%. With the introduction of RDTs, this increased to 99.3% in 2015, with a mean test positivity rate of 42% during the 15 year period. The proportion of all consultations due to malaria decreased from 39.7% in 2001 to 26.9% in 2007 (pre-RDT). After the introduction of RDTs and change in definition from clinical to laboratory-confirmed, the proportion of all consultations due to malaria decreased from 9.1% in 2008 to 5.0% in 2015. The proportion of patients hospitalized for malaria accounted for 11.5% of all hospitalizations in 2001, and decreased to 7.5% in 2015. From 2001 to 2007, the proportion of hospitalized deaths attributable to malaria decreased from 24.5% in 2001 to 8.1% in 2007. From 2008 to 2015, the proportion of hospitalized deaths attributable to malaria decreased from 7.1% to 3.5%. We conclude that malaria morbidity and mortality has decreased substantially during the scale up of malaria control interventions.

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ASSOCIATION BETWEEN HOUSE QUALITY AND MALARIA INFECTION IN SUB-SAHARAN AFRICA: A MULTI-COUNTRY ANALYSIS

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Improvements to housing may contribute to malaria control and elimination by reducing house entry by malaria vectors and thus exposure to biting. We tested the hypothesis that malaria infection risk is lower in modern, improved housing compared to traditional housing in sub-Saharan Africa (SSA). All Demographic and Heath Surveys (DHS) and Malaria Indicator Surveys (MIS) that measured malaria infection by rapid diagnostic test or microscopy in SSA were analysed. Houses built using non-rudimentary wall, roof and floor materials were classified as modern and all other houses were classified as traditional. Conditional logistic regression was used to determine the association between house quality and prevalence of malaria infection in children aged 0-5 years, adjusting for age, gender, intervention coverage, household wealth and geographic cluster. We will present the association between house quality and the odds of malaria infection in children and discuss the potential of improved

housing as an intervention against malaria in a range of transmission settings across SSA. Improved housing may be an important strategy to prevent the re-introduction of malaria in areas where malaria has been eliminated.

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NOVEL APPLICATION OF A SPATIAL ANALYSIS METHOD IDENTIFYING FINE-SCALE SPATIAL CLUSTERING OF MALARIA DISEASE AND MOSQUITO VECTORS AMONG MATCHED CASES AND CONTROLS IN BLANTYRE, MALAWI

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Matching cases and controls in epidemiological studies usually produces greater statistical power in analyses, but precludes direct analysis of matched variables on the outcome. We analyzed clinical cases of malaria in under-five year olds in Blantyre Malawi (n=187) with age-, and residence-matched controls (n=286) who were *Plasmodium*-negative from the same clinic. To explore spatial patterns of household environmental risks among malaria cases and vectors, we used nonhomogeneous Poisson point process (NPPP), an analytic method employed in landscape ecology, which treats the cases as a 'presence-only' data set and omits controls from analysis, to determine environmental variables associated with malaria case or anopheline-positive households. Using this method, non-random spatial distributions of case ($p<0.01$) and anopheline-positive households ($p<0.01$) were detected. In a 'pseudo-absence' logistic regression model informed by NPPP results, environmental variables associated with both malaria case- and anopheline-positive households included natural roofs ($p<0.01$), unfinished walls ($p=0.02$), open eaves ($p=0.02$), and proximity to open water ($p=0.02$). NPPP is a method that can expand the use of matched case-control data that has been matched on location, although there is a cost in power related to diminished sample size. These results demonstrate that there are environmental factors associated with increased risk of malaria in urban and peri-urban Blantyre.

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SCALING-UP AND USING ROUTINE MALARIA SURVEILLANCE DATA TO IDENTIFY MALARIA HOTSPOTS AND TARGET MALARIA CONTROL INTERVENTIONS DURING AND AFTER THE EBOLA EPIDEMIC IN GUINEA

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Malaria is the principal cause of morbidity and mortality in Guinea; its burden was exacerbated by the recent Ebola outbreak. Due to historical limitations in the routine health management information system, the Guinea National Malaria Control Program (NMCP) developed a monthly reporting system for malaria epidemiologic and commodity data prior to the outbreak. With the expansion of access to rapid diagnostic tests for malaria, the reporting system has facilitated accurate tracking of malaria burden through widespread diagnostic confirmation of cases. The scale-up of this system also coincided with the Ebola outbreak and the related interruptions in health care service delivery in Ebola-affected areas. The malaria reporting system is based on standardized forms completed at health facilities. Data are entered into the electronic system at the district level and analyzed by the NMCP. The NMCP issues a monthly malaria bulletin that is disseminated nationally and regionally. The bulletin reports malaria incidence, testing and treatment rates, and commodity stock levels for Guinea's 38 health districts. Each bulletin also highlights the 10 health facilities reporting the highest incidence of malaria cases. Completeness of data entered at the district level increased from 66% for the first issue in November 2014, to 82% by March 2015, to 97% in January 2016. As data completeness improved, the bulletin allowed the NMCP to identify a high malaria transmission hotspot in Boffa district, where annualized malaria incidence in certain areas was as high as 425 cases per 1,000 population. Follow-up investigations based on surveillance data allowed NMCP and district health officers to target behavior change communications to hotspot areas. Despite staffing and logistics challenges related to the Ebola outbreak, the NMCP was able to successfully scale up a new malaria-specific reporting system. This system allowed the NMCP to improve data reporting from remote areas and to begin using routine surveillance data to identify malaria hotspots and target interventions.

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AN OUTBREAK OF AUTOCHTHONOUS MALARIA IN THE ATLANTIC FOREST, STATE OF RIO DE JANEIRO, BRAZIL

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In the state of Rio de Janeiro (RJ) malaria transmission was considered eliminated in 1968 but since 1993 some sporadic autochthonous cases with history of having visited native locations of the Atlantic Forest (AF) are been described with no identifiable source of infection. From 2006 to 2014, 51 autochthonous malaria cases were reported, an average of 5.6 cases/year. We describe an outbreak of autochthonous malaria that occurred in 2015 in the AF of RJ state diagnosed in Fiocruz. A FOUR-fold increase over the average of autochthonous malaria cases from RJ, with 25 individuals without history of travelling outside the State being diagnosed with malaria infection. Most male (23/25) aged 7-59 years (median 41y), with clinical presentation characterized by sudden onset of fever, chills, headache, and myalgia. The time from onset of symptoms and diagnosis varied from 3 to 21 days (median 13 days). The fever pattern was daily progressing to a tertian pattern after a median of 10 days. Microscopic examination (thick blood smear) showed low parasitaemia (48 p/mm3 to 1200 p/mm3) with unusual morphological forms of *Plasmodium vivax*. All cases were positive for *P. vivax* by PCR, and this is currently been investigated by genomic sequencing. No patient presented clinical or laboratory complications and all were treated with chloroquine and primaquine, as recommended by Brazilian guidelines. Most (22/25) were inhabitants of RJ capital city, who visited AF areas for leisure or work-related activities in vegetation-dense areas (median 8 days). The median time between probable exposure and onset of symptoms was 24.5 days (range: 17 to 41). No individual had visited well-established malaria endemic areas or had any other risk factor for infection. The presumed areas where the infections occurred are widespread throughout the AF with mountainous topography and very dense native vegetation coverage, a natural habitat for malaria vectors *Anopheles* (Kerteszia), and with circulation of non human primates. This indicates that the transmission can occur, and may reflect the existence of a zoonotic transmission cycle.

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BREAKDOWN IN MALARIA CONTROL IN VENEZUELA - 440% INCREASE IN MALARIA CASES

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Venezuela's health care system is in deep crisis at all levels. Patients in urban and rural areas do not have access to essential drugs and prevention activities have been severely affected. The country is experiencing a breakdown in malaria control and national health authorities stopped reporting malaria epidemiological information in November 2015. The routine weekly malaria reporting system started in the 1960s and plays a key role in the identification of malaria epidemics and it is a useful tool for decision-making at the national malaria program. The objective of this study was to describe the changes in the total number of confirmed malaria cases (officially reported) between 2000-2015 and compared them with the adjusted malaria estimations for the same period. Malaria morbidity data was updated incorporating information from the Venezuelan health system including annual national epidemiological bulletin, the world malaria report 2015, and data on relapses, recrudescences, under reporting and self-treatment rates. Between 2000-2015, Venezuela officially reported 794,531 cases of malaria contrasting with the estimated 1,172,285 malaria cases for the same period. Compared to the baseline in 2000, in 2015 Venezuela had a 350% and 440% increased of reported and estimated malaria cases respectively. This represent approximately 25% of the malaria cases in the Americas. 80% of Venezuelan's malaria morbidity is concentrated in Sifontes Municipality, Bolivar State a complex operating environment with high mobilization, crime rates, illegal mining and lack of security. Malaria interventions are limited in the country: there is not mass/routine distribution of bednets, stock out of antimalarials are common, no malaria rapid test are routinely used and logistical/administrative limitations impedes the implementation of the activities. It is unjustifiable that in a country that has a trillion dollars from oil revenues in the last decade has derailed its health's investment to protect its population from malaria. There is a need to act now to prevent further spread of this malaria epidemic in Venezuela and neighboring countries.

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EFFECTS OF SEASONALITY AND HOST FACTORS ON LONGITUDINAL *PLASMODIUM FALCIPARUM* GAMETOCYTE PREVALENCE IN KENIEROBA, MALI

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In order to build upon recent gains in global malaria control and elimination efforts, it is necessary to enhance our current understanding of the spatiotemporal dynamics of parasite carriage and the human risk factors affecting them in areas with varying transmission patterns and intensities. To achieve this objective, sensitive molecular diagnostic tools have been recently developed and applied in epidemiological surveys of both *Plasmodium falciparum* blood-stage parasites and sexual-stage gametocytes in endemic areas. However, many of these studies are cross-sectional by design and fail to effectively address the longitudinal dynamics of gametocytes that are critical for transmission. In Kenieroba, Mali, where malaria transmission follows a seasonal pattern, we conducted a one-year longitudinal cohort study and used molecular methods to assess the dynamics of both total parasite and gametocyte prevalence among village residents. From June 2013 to May 2014, we followed a cohort of 500 individuals aged 1-65 years that represented the age structure of the village population, and measured both *P. falciparum* parasitemia (PCR) and gametocyte prevalence (stage-specific RT-PCR) in peripheral blood every two weeks. Approximately 80% of *P. falciparum* DNA-positive individuals were found to be gametocyte-positive, regardless of the time of year. In addition to asexual parasite prevalence, host age (peak at 9-16 years) and gender (higher in males) were also significantly associated with longitudinal gametocyte prevalence. Among *P. falciparum*-positive individuals, longitudinal gametocyte prevalence over the one-year period was found to be consistently high (median range: 72-82%) among children aged 16 years or less, and then declined with increasing age. Other host factors (i.e., G6PD genotype, ABO blood type, and sickle-cell trait) showed no significant association with longitudinal gametocyte prevalence. Our findings show that asexual parasite prevalence, and host age and gender are important determinants of longitudinal gametocyte prevalence in a seasonal, high-transmission area of Mali.

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THE EFFECT OF HOLES IN LONG-LASTING INSECTICIDAL NETS ON MALARIA: A CASE-CONTROL STUDY IN MALAWI, 2013

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Long-lasting insecticidal nets (LLINs) are a cornerstone of malaria prevention. Holes develop in LLINs over time, which compromise their physical integrity; however, the effect of holes on the risk of malaria infection is not well known. After a mass LLIN distribution in southern Malawi in 2012 that led to >95% LLIN coverage among children, we conducted one of the first in-depth studies to assess the relationship between LLIN holes and malaria. From March–September 2013, we enrolled febrile children ages 6–59 months who consistently slept under LLINs (every night for two weeks before illness onset) in a case-control study at a clinic. Cases were positive for *Plasmodium* by microscopy, and controls were negative. Digital photographs of participants' LLINs were taken and analyzed using image processing software to measure holes. Total hole area was divided into quartiles and the World Health Organization's (WHO) proportionate Hole Index (pHI) cut-offs: <79 cm^2 (good), 80–789 cm^2 (damaged), and >790 cm^2 (too torn). We compared hole characteristics between case and control LLINs using non-parametric and logistic regression analyses. Of 248 LLINs analyzed, 97 (39%) were from cases. Overall, 86% of LLINs had at least one hole. The median number of holes per net was 9 for case and control LLINs (p=.82). Hole location was divided into roof, upper halves, and lower halves. For both case and control LLINs, the median number of holes in the roof was 0 and in the upper halves was 2. The median number of holes in the lower halves of LLINs was 6 for cases and 7 for controls (p=.63). Median total hole area was 10 cm^2 for controls and 8 cm^2 for cases (p=.10). Multivariate modeling showed no association between pHI or total hole area quartiles and malaria, controlling for child age, caregiver education, socioeconomic status, windows, closed versus open housing eaves, and iron versus thatched roof. LLINs in this study were in relatively good condition one year after an LLIN campaign, which may be why holes were not associated with increased odds of malaria. Future studies should examine associations between LLIN holes and malaria risk in other populations and with more damaged nets.

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INTER-PROVINCIAL DIFFERENCES IN MALARIA CASE MANAGEMENT PRACTICES IN ANGOLA: A CROSS-SECTIONAL HEALTH FACILITY SURVEY

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Malaria accounts for the largest portion of healthcare demand in Angola. A pillar of malaria control in Angola is the appropriate management of malaria illness, including testing of suspect cases with rapid diagnostic tests (RDTs) and treatment of confirmed cases with artemisinin-based combination therapies (ACTs). Periodic systematic evaluations of malaria case management are recommended to measure health facility (HF) readiness and adherence to national case management guidelines. Cross-sectional HF surveys were performed in low-transmission Huambo and high-transmission Uíge Provinces in early 2016. In each province, 45 HFs were randomly selected from among all public HFs. Survey teams performed inventories of malaria commodities and conducted exit interviews and re-examinations, including RDT testing, of a random selection of all patients completing outpatient consultations. Key HF readiness and case management indicators were calculated adjusting for the cluster sampling design. Availability of RDTs on the day of the survey was 71% (54-83) in Huambo and 85% (67-94) in Uíge. At least one formulation of an ACT was available in 83% (66-92) of HFs in Huambo and 79% (61-90) of HFs in Uíge. A total of 590 patients in Huambo and 634 in Uíge were re-examined. Among re-examined patients in Huambo, 8.9% (95% CI: 5-15) were true malaria cases, compared to 32% (26-39) in Uíge. Testing rates of suspect malaria cases in Huambo were 31% (23-39) versus 70% (55-82) in Uíge. Overall, 28% (13-50) of patients with uncomplicated malaria, as determined during the re-examination, were appropriately treated with an ACT with the correct dose in Huambo, compared to 62% (44-77) in Uíge. The results reveal important differences between provinces. Despite similar availability of RDTs and ACTs, testing and treatment rates were significantly lower in Huambo compared to Uíge. A majority of true malaria cases seeking care in HFs in Huambo were not appropriately treated with antimalarials, highlighting the importance of continued training and supervision of healthcare workers in malaria case management, particularly in areas with decreased malaria transmission.

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CLINICAL MALARIA INCIDENCE RATE COLLECTED DURING MALARIA TRANSMISSION BLOCKING VACCINE STUDY IN ADULTS VOLUNTEERS IN BANCOUMANA, MALI

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Malaria control strategies have focused on children under the age of 5 years and pregnant women. However, more studies in West Africa showed that clinical malaria attacks also occur in adults living in areas of high endemicity. The present study was undertaken to investigate the clinical malaria incidence in adults living in malaria endemic area. A total of 120 and 200 volunteers aged from 18 years to 50 years old were enrolled respectively in malaria vaccine study of Pfs25-EPA/Alhydrogel® and of combined Pfs230D1M-EPA/Alhydrogel® and Pfs25-EPA/Alhydrogel® in Bancoumana, Mali. Clinical malaria data were collected from May 2013 to December 2014 and from May 2015 to December 2015 respectively in study cohort of Pfs25-EPA/Alhydrogel® and of combined Pfs230D1M-EPA/Alhydrogel® and Pfs25-EPA/Alhydrogel®. Malaria smear and or rapid diagnostic test (RDT) was performed in case of clinical symptoms to confirm clinical malaria before the treatment initiation. In 2013, from July to December, the incidence rate of clinical malaria during that transmission season period was 0.96 (101/110) episode of malaria per person per season. In 2014, during the same malaria transmission season period, the incidence rate of clinical malaria was 0.87 episode of malaria per person per season (94/107), during that year, the overall incidence rate of clinical malaria was 0.99 (106/107) episode per person per year. In 2015, from July to December, the incidence rate of clinical malaria during that transmission season period was 0.91 (174/191) episode of malaria per person per season. During that year, the overall incidence rate of clinical malaria was 0.96 (185/191) episode per person per year. The incidence of malaria continues to be high and seasonal in Bancoumana in adults population. This malaria burden in adult population needs to be taken into account in malaria control strategies.

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GENETIC POLYMORPHISM OF MEROZOITE SURFACE PROTEIN2 (MSP2) IN *PLASMODIUM FALCIPARUM* ISOLATES FROM PAWE DISTRICT, NORTHWEST ETHIOPIA

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In malaria-endemic regions, *Plasmodium falciparum* infection is characterized by extensive genetic diversity. Describing this diversity provides important information about the local malaria situation. This study was conducted to evaluate the extent of genetic diversity of *P. falciparum* in Pawe, in the northwest Ethiopia. A total of 92 isolates from patients with uncomplicated *P. falciparum* attending Pawe Health Centre was collected from September to December, 2013.Genomic DNA was extracted using Chelex® method and anlysed by length polymorphism following gel electrophoresis of DNA products from nested-PCR of msp2 (block 3) targeting allelic families of FC27 and 3D7/IC. There were 22 different *MSP2* alleles, 11 corresponding to the3D7/ IC/ and 11 to the FC27 allelic family. However, isolates of the 3D7/IC allelic family showed

higher frequency (87%) compared to FC27 (85%). The overall multiplicity of infection was 2.8 (CI 95% 2.55-3.03), seventy-six percent of isolates contained multiple infections. The heterozygosity index was 0.75 for msp2 .There was no statically significant difference in the multiplicity of infection by either age or parasite density. In conclusion, this study showed that the genetic diversity in *P. falciparum* isolates from northwest Ethiopia was high and mainly of multiple infections.

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ROLE OF ALPHA-THALASSEMIA AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN *PLASMODIUM FALCIPARUM* TRANSMISSION FROM HUMAN TO MOSQUITO

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Large evidence is available showing that human genetic variation affects susceptibility to infectious diseases, but it is unknown whether it also affects the host efficiency to transmit pathogens. We have previously shown that haemoglobin S and C, known to protect from clinical *Plasmodium falciparum* malaria, increase the transmission of the parasite from the human host to the mosquito vector. In this study we evaluated the role of 3.7 alpha deletional thalassemia and glucose-6-phosphate dehydrogenase deficiency, in the ability to infect mosquitoes.To assess the impact of these genetic factors on the ability to infect mosquitoes, we conducted Standard Membrane Feeding Assays on blood samples from 69 children aged 3-15 years from the village of Soumousso, Burkina Faso with known alpha thalassemia and G6PDA- genotypes. A total of 15515 *Anopheles* were dissected on day seven after membrane feeding and oocysts were detected by microscopy in mosquito guts.We found that alpha thalassemia increases both the prevalence and density of *P. falciparum* infection in mosquitoes while G6PDA- increases the prevalence but not the density of infection.These results confirm that human genetics variation affects *P. falciparum* transmission from human to mosquitoes.

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GENETIC DIVERSITY AND COMPLEXITY OF *PLASMODIUM FALCIPARUM* ISOLATES IN NORTH-CENTRAL NIGERIA

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Malaria is a parasitic disease of significant public health importance in Nigeria. Population-specific investigation of the genetic diversity of the parasites is important for effective vaccinological and chemotherapeutic intervention. This study determine the genetic diversity of *Plasmodium falciparum* isolates using two antigenic markers in individuals attending health facilities in Idah and Ibaji Local Government Areas of Kogi State, North-Central, Nigeria. DNA was extracted from finger-prick blood samples collected from *P. falciparum* positive individuals, followed by polymerase chain reaction genotyping which targeted the MSP-1 and MSP-2 allelic families. All the three families of MSP-1 (K1, MAD20 and RO33) and two of MSP-2 (FC27 and 3D7) were observed among the isolates. Prevalence of (MSP-1) K1, MAD20 and RO33 were 70%, 20% and 40% respectively for Idah while prevalence of 40%, 10% and 20% was recorded for MSP-1 (K1, MAD20 and RO33) respectively. Analysis of MSP-2 allelic families revealed prevalence of FC27 family was 40% in Idah and 30% in Ibaji while 3D7 had a prevalence of 20% in both Idah and Ibaji. The frequency of FC27 genotypes was higher than 3D7 in both populations. Multiplicity of Infections (MoI) for MSP-1 was higher in Ibaji (1.30) than Idah (1.05) while MoI with MSP-2 families was lower in Ibaji (2.00) than Idah (2.13). There was no significant difference between the MoI values in Idah and Ibaji ($P > 0.05$). The expected heterozygosity (HE) value was 0.56 for MSP-1 and 0.84 for MSP-2 showing higher diversity in MSP-2. The findings showed a high genetic diversity of *P. falciparum* in the study populations providing population-specific genetic information on the malaria parasites circulating in North-Central Nigeria.

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INFLUENCE OF GENETIC AND EPIGENETIC VARIATIONS ON MALARIA SUSCEPTIBILITY

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Erythrocyte phase of *Plasmodium falciparum* malaria infection results from complex membrane sorting and signaling. Proteins in the erythrocyte membrane lipid rafts regulate membrane sorting and signaling processes in erythrocytes, and hence some of these proteins (Gαs and ß2AR) and their interacting proteins (ADORA2A and GRK5) were expected to influence pathogen entry to erythrocytes. It is also known that entry of malaria parasite in patients induces the synthesis of inflammatory cytokines such as TNF-α, IL-1, IL-6 and IL-10. Altered gene expression of cytokines regulated by mutations or epigenetic mechanisms, and cytokines by themselves inducing epigenetic changes may influence malaria pathogenesis. Epigenetic modifications of drug transporters like P-glycoprotein may also influence the chemotherapy for malaria. We hypothesize, that genetic and epigenetic variants in GNAS, ADRB2, ADORA2A, GRK5 and ABCB1 genes may influence the malaria susceptibility. To test the hypothesis, a case - control study of individuals affected by *P. falciparum* malaria versus healthy controls, was performed. Genetic and/or epigenetic variations of the genes were analyzed after PCR-RFLP and direct DNA sequencing. Western blotting was used to access the levels of ABCB1 protein. Genetic association was observed at allele, genotype and haplotypes of SNPs in the genes, and significant DNA methylation differences among cases and controls. Our study provides evidence for the proposed role of studied genes mediated mechanisms in the etiology of malaria susceptibility.

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K13 POLYMORPHISMS IN *PLASMODIUM FALCIPARUM* FROM LOW AND HIGH TRANSMISSION AREAS IN KENYA

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The development of artemisinin resistance in Southeast Asia (SEA) threaten malaria control globally. The recent genetic marker Kelch 13 propeller has proven useful in identifying resistance but only in SEA. Sub-Saharan African (SSA) countries show mutations in this marker but none of the haplotypes are those reported in SEA. Further, none of the observed mutations in SSA exhibit delayed parasite clearance, defined as longer than 3 days following treatment with artemisinin. On the Kenya's coast, a study showed a decelerated responsiveness to ACTs but it was not clear whether it was due to declining immunity or changes in parasite sensitivity. With this realization it is important for SSA countries to identify single nucleotide polymorphisms (SNPs) that would best describe resistance in this part of the world. A total of 380 *Plasmodium falciparum* clinical isolates, collected before 2003 and after the introduction of artemisinin combination therapy, were screened for Kelch 13 propeller mutations. These were collected from regions with different malaria transmission intensities in Kenya which include Kisumu, Kombewa, Marigat, Kericho, Kisii and Malindi. Twenty six pre-ACT isolates screened showed mutation at position 493 (7.7%) and 539 (7.7%). Mutations in both these positions

have been reported in SEA and have shown delayed parasite clearance 3 days post treatment. Mutation at position A578S accounted for 3.8% of the mutations. This mutation has been reported in Bangladesh and SSA and is said to alter protein interactions although not reported to have any delayed parasite clearance following treatment with ACTs. Unique SNPs were also found with D464N accounting for 15% of the mutations. There is an urgent need for continued identification of ACT resistance markers for SSA parasites, in part to ease traditional laborious surveillance efforts and to aid in prompt action in the event of identified ACT resistance.

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GAMETOCYTE CARRIAGE IN A LOW MALARIA TRANSMISSION AREA OF GHANA

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Gametocytes (the sexual stage parasites of *Plasmodium falciparum*) play a major role in malaria transmission in different endemic settings and their identification is important for transmission-blocking interventions. Asymptomatic individuals in malaria endemic areas tend to harbor both microscopic and submicroscopic gametocytes of *P. falciparum* which can infect mosquitoes and contribute to malaria transmission. Knowledge of the population at risk of harboring gametocytes is important in determining the infectious reservoir for transmission-blocking interventions. This cross-sectional study therefore aimed to determine the prevalence of *Plasmodium falciparum* gametocytaemia among a cohort of children and its association with the transmission pattern in a low malaria transmission area of Ghana. A total of 181children within age groups 2-5, 6-10 and 11-15 years took part in this study. Finger prick and venous blood samples were taken from the children and screened for the prevalence of *Plasmodium falciparum* parasites by microscopy, Nested PCR targeting Plasmodium DNA and Real-Time PCR targeting both 18SrRNA gene and transcripts using the gametocyte marker pfs25. Data collected were analyzed using R regression and GraphPad Prism 5. Out of 181 children, 3.9% (7/181) had microscopically confirmed asexual parasites, with no gametocytes found (0/180). Asexual parasite prevalence was 4.4% (2/48), 2.5% (2/80) and 5.8% (3/53) for 2-5, 6-10 and 11-15 years respectively. nPCR gave an overall parasite prevalence of 7.2% (13/181) with prevalences of 1.1% (2/181), 2.2% (4/181) and 3.9% (7/181) for ages 2-5, 6-10 and 11-15 years respectively. qPCR showed 6 out of 13 nPCR positive samples had gametocytes with one sample missing. Within the age groups, individuals carrying gametocytes were 1, 3 and 3 for ages 2-5, 6-10 and 11-15 years respectively. The study has therefore determined the presence of submicroscopic gametocytaemia in the cohort and this is likely to contribute to malaria transmission in the study area.

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GENETIC DIVERSITY AND NATURAL SELECTION AT DOMAIN I OF *PLASMODIUM FALCIPARUM* APICAL MEMBRANE ANTIGEN 1 NIGERIAN ISOLATES: RELATIONSHIP WITH T-CELL RESPONSES OF MALARIA PATIENTS FROM LAGOS NIGERIA

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The *Plasmodium falciparum* apical membrane antigen I (AMA1) is a leading malaria vaccine candidate antigen. Population genetic analyses of vaccine candidate antigens provide insights into the status and natural dynamics of diversity and evolution in these antigens. T-cell responses mediate immunity against malaria. This study describes the extent of genetic polymorphisms and selection at the hyper-variable domain I of AMA1 among *Plasmodium falciparum* isolates circulating in the Nigerian population and the consequence on T-cell responses of malaria patients. The Domain I of AMA1 gene was amplified in a nested-PCR and sequenced in both directions from 195 P.falciparum isolates collected from microscopically confirmed P.falciparum dry blood spots of patients from the three senatorial districts in Lagos, Nigeria. Pro-inflammatory cytokines were determined by capture ELISA. A total of 74 AMA1 haplotypes were observed among 195 isolates sequenced. Forty-eight of these 74 haplotypes are new and here reported for the first time. The nucleotide diversity (ϖ) was in the order Ajeromi > Ijede > Lekki while the number of haplotypes (H) was highest in Ajeromi with relatively higher transmission. Analysis of the inter-population genetic differentiation showed moderate gene flow and genetic differentiation (Fst range = 0.007-0.037) between two populations. Analysis of the non-synonymous and synonymous mutations, Tajima's D and recombination rates showed evidence of positive selection in the AMA1 antigen with high rates of recombination events while Phylogenetic analysis showed no population-wise clustering. The relationship between genetic diversity in AMA1 and innate immune responses revealed no statistically significant association. In Nigeria, PfAMA1 is under positive natural selection with 48 new haplotypes reported. Genetic diversity level and selection were highest in Ajeromi, the gene flow among these populations were moderate. T-cell components of immunity against malaria are independent of genetic polymorphisms in AMA1. Data reported here will update information needed for the development of effective malaria vaccine.

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GENETIC STRUCTURE OF *PLASMODIUM FALCIPARUM* ISOLATES IN PRE-ARTEMISININ THERAPY ERA OF INDIA

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Population studies conducted on *Plasmodium falciparum* have revealed its success to perpetuate high genetic diversity and this diversity has hampered all attempts to control malaria. Limited information is available about genetic sub-structuring in Indian falciparum populations. We want to evaluate the genetic diversity and population structure prevailed in high and low falciparum prevalent malaria endemic areas, and to predict the geographic origin of Indian falciparum malaria before artemisinin regimen. In this study, samples were collected from six study sites from both low and high falciparum prevalent areas across India. The samples were collected in year 2002-2006, when artemisinin was not introduced as antimalarial treatment in India. Twelve polymorphic microsatellite markers were used to understand the genetic structures of falciparum populations. All the parasite populations were analyzed for genetic diversity, linkage disequilibrium and genetic structure. The measures of genetic diversity revealed all microsatellite loci to be polymorphic and the number of alleles per locus varied from 4 to 14. The mean expected heterozygosity (He) were from 0.376 ± 0.036 and 0.864 ± 0.039, revealing a moderate to high level of genetic diversity at these loci. Evaluation of geographic population structure within and among populations using F-statistic and STRUCTURE analysis revealed genetically distinct groups in accordance with transmission intensity of different geography. Results of this study will provide an insight towards the population structure of falciparum prevailed before artemisinin regimen in India and also provide a basis to study how artemisinin could affect the population structure in Indian falciparum population.

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DOES A SINGLE PERIPHERAL BLOOD SAMPLE FROM A MALARIA-INFECTED INDIVIDUAL CAPTURE ALL PARASITE GENOTYPES PRESENT IN AN INFECTION?

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There is contradicting data about whether or not a single peripheral blood sample accurately captures all parasite genotypes present in a malaria infection. Previous studies have demonstrated a rapid turnover of parasite genotypes during the course of an asymptomatic infection with some genotypes appearing while others disappearing from the peripheral blood. This rapid turnover of genotypes suggests that a peripheral blood sample taken at a single time-point contains only a subset of parasite genotypes present in the entire malaria infection. Parasite genotypes not detected in the peripheral blood are thought sequester in deep tissues precluding their detection in the peripheral blood. However, recent studies have shown that parasites sequestered in deep tissues are genetically identical to those circulating in the peripheral blood. This suggests that a single peripheral blood sample effectively captures all the parasite diversity present in the infection. These discrepant findings may have resulted from the poor resolution of msp1/2 genotyping methods used to determine the genetic composition of infections. To resolve problems associated with msp1/2 measures of infection complexity, we have employed a more-sensitive and less ambiguous 24-SNP Taqman assay to obtain the DNA fingerprint of malaria parasites sampled from adults with asymptomatic malaria over the course of seven consecutive days. We have used this approach to examine whether or not the within-host parasite genetic diversity in asymptomatic individuals remains constant or changes over seven consecutive days. Results from the first set of asymptomatic infections will be presented and discussed at the meeting.

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COMPARISON OF TWO GENOTYPING METHODS FOR DISTINGUISHING RECRUDESCENCE FROM RE-INFECTION IN ANTIMALARIAL DRUG EFFICACY/EFFECTIVENESS TRIALS

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In areas of intense malaria transmission, individuals treated for malaria may encounter new episodes of malaria parasitaemia during the period of follow-up. Without comparing the genetic identity of pre-treatment and post-treatment parasites, it is difficult to resolve whether the recurrence is as a result of treatment failure (recrudescence) or a new infection. Genotyping of the merozoite surface proteins 1 and 2 (msp 1 and 2) is the current gold standard for genotyping infections to correct drug efficacy/effectiveness data. However interpretation of msp1 and msp2 data is often ambiguous and subjective. Therefore, new and better methods for distinguishing recrudescence from re-infection are urgently needed. We compared the performance of the msp1 and msp2 genotyping with a high sensitivity and high resolution 24 single nucleotide polymorphism (SNP) Taqman assay in a cluster-randomized effectiveness trial in an area of high malaria transmission in Malawi. Filter paper samples were collected on day 0 and day 42 of follow-up from children with malaria aged 4-11 (n=106) treated with either artemether-lumefantrine or dihydroartemisinin-piperaquine. Parasite DNA was extracted from pre-treatment (day 0) and post-treatment (day 42) and genotyped using msp1 and msp2 genotyping method and 24-SNP Taqman assay as previously described.The agreement between the two methods was 86%. Discordant results were often due to false positive msp1/2 results. We found a high rate of re-infection with 33% of new episodes of parasitemia detected on day 42 of follow up. Rate of treatment failure based on SNP barcoding of day 0 and d42 filter paper blood samples was 3%. A full comparison of the two genotyping methods for distinguishing re-infections and treatment failure will be presented during the meeting.

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MICROSATELLITE ANALYSIS REVEALS DIFFERENT TRANSMISSION PATTERNS IN THE PERUVIAN AMAZON

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Population genetics studies provides critical information about where and when the infection took place, so imported cases as result of migration can be separated from endogenous cases that indicate the efficacy of the control programs. To address the hypothesis that endogenous transmission is the main mechanism that maintain malaria in rural communities in Peruvian Amazon, 1031 *Plasmodium vivax* positive samples were identified from a population-based cohort by PCR performed every three months from March 2013 to March 2014 in two communities: Cahuide (CAH), community with high mobility by road; and Lupuna (LUP), riverine and isolated community. 390 samples were chosen for microsatellite (MS) genotyping with 9 previously reported MS and 7 new MS. High genetic diversity was observed among communities (He 0.622 ± 0.045) but it was low (0.38 ± 0.043) in LUP in December 2013. Higher proportion of polyclonal infections was found in CAH (19%-36%) in comparison to LUP (5%-24%). AMOVA analysis showed that most of the variance occurs within population (58%) and among communities (37%), suggesting strong population structure within and between communities. Genetic differentiation was high between communities, but low between months in each community (Pairwise FST 0.039), except for LUP in March 2013 which showed moderate differentiation with respect to other months in LUP (0.25), but low differentiation in comparison to CAH (0.09). Analysis of population structure revealed the presence of 4 clusters or subpopulation within these communities (Cluster A, B1, B2 and C).Cluster A was mainly present in March and June 2013 in CAH and only in March 2013 in LUP. Cluster C was predominantly along the follow-up in LUP, except in March 2013. Neighbor joining and burst analysis showed a clonal expansion of these two clusters. Regarding clusters B1 and B2, they are polyphyletic groups that did not maintain fixed over time. In conclusion, results are consistent with an outbreak in Cahuide caused by a clonal expansion of cluster A. In contrast, Lupuna showed endogenous stationary transmission pattern caused by Cluster C that prevails along the seasons.

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POPULATION GENETICS OF *PLASMODIUM VIVAX* IN MICROSCOPIC AND SUB-MICROSCOPIC INFECTIONS IN RIVERINE AND ROAD COMMUNITIES

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Plasmodium vivax population diversity has been reported along time in different settings in the Peruvian Amazon. The aim of this study was to explore the genetic diversity and parasite population structure of *P. vivax* parasites from microscopic and submicroscopic infections in two communities: Cahuide (Km 56 at Iquitos-Nauta road) and Lupuna (accessible only by river), in Loreto. 473 *P. vivax* positive samples were selected from a cohort of 1031 *P. vivax* positive samples based on DNA quantity that will allow Microsatellite (MS) analysis. These samples were collected from March 2013 to March 2014 (168 samples from Cahuide and 305 samples from Lupuna) by monthly active case detection surveys. All samples were confirmed as positive to *P. vivax* by microscopy and/or PCR, DNA was quantified by qPCR. Only 437 samples were used for genotyping using 16 microsatellites. Genotyping was performed and subsequently analyzed by capillary electrophoresis using an ABI PRISM 3100 avant genetic analyzer. Based on microscopy and/or PCR, from the total number of samples, 177 (35%) were classified as submicroscopic and 296 (65%) as microscopic. From genotyped samples, 339 (77%) were classified as monoclonal infections and 98 (29%) were polyclonal infections. Around 361 had an almost-complete allele profile (75% or higher). Our results showed an average genetic diversity (He) of 0.565 for Cahuide and 0.52 for Lupuna, meaning high genetic variability for both communities. A total of 51 haplotypes were found, 21 haplotypes were present in Cahuide and 30 in Lupuna. 2 haplotypes with the highest frequencies where found one in each community. In Cahuide, "haplotype-20" is present only during March and June 2013 in 23 samples; whereas in Lupuna "haplotype-42" is present along year 2013. Interestingly 4 population clusters were found in both communities with no differentiation between microscopic and submicroscopic infections. This data suggests that there is no genetic differences between parasites from microscopic and submicroscopic infections and are stable along time. Population structure of *P. vivax* might be explained by other factors rather than type of infection.

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VALIDATING A SNP-BASED BARCODING TOOL FOR *PLASMODIUM VIVAX* IN PAPUA NEW GUINEA

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Plasmodium vivax is one of the four species of Plasmodium that cause malaria in Papua New Guinea (PNG) and is the dominant species in some areas of PNG. Efforts to reduce and eventually eliminate malaria in PNG will require a combination of different strategies and tools to effectively monitor the parasite. We have validated a field deployable SNP-based barcoding tool for genotyping *P. vivax* parasites in PNG. We selected *P. vivax* field samples with infections containing single parasite clones for validating this new tool. The selection of single clone infections was based on genotyping length polymorphic molecular markers, Pvmsp1F3 and microsatellite MS16. We then performed High Resolution Melting (HRM) analysis on amplicons from established polymerase chain reaction assays. Genotyping was performed on 24 SNPs, which were previously identified amongst a global panel of isolates. These SNPs are spread across all 14 chromosomes of *P. vivax* and are located on putatively neutral sites. Eight of the 24 SNPs had a minor allele frequency of ≥0.15 across three different geographic areas of PNG. These were identified as suitable candidates for genotyping *P. vivax* parasites in PNG. A complete 8-SNP-haplotype was obtained for 42 (44%) of the 96 samples tested. Amongst these there were 33 unique haplotypes identified. The discriminatory power of SNP-based barcoding will be compared with that of the fragment length polymorphic markers which are currently being used to genotype *P. vivax* in PNG. SNP-based barcoding is a field based tool that can be adapted to platforms that perform HRM and with further validation may be useful for studying *P. vivax* population genetics in PNG.

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LONGITUDINAL POOLED DEEP SEQUENCING OF *PLASMODIUM VIVAX* KELCH PROPELLER DOMAIN IN CAMBODIA REVEALS A LACK OF DIRECTIONAL SELECTION

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The emergence of artemisinin resistance among *Plasmodium falciparum* in the Greater Mekong region threatens malaria treatment and control interventions. Mutations in K13 (PF3D7_1343700) provide potential molecular markers of artemisinin resistance in *P. falciparum*. The aim of this study was to survey the sympatric species, *P. vivax*, in Cambodia, for mutations in the orthologous gene (K12, PVX_083080) that might similarly confer artemisinin resistance. 359 clinical isolates collected in western and northern Cambodia from 2009-2013 were organized into eleven pools by province and year. The propeller domain of PVX_083080, 2139 bp in length, was amplified using PCR. Amplification products were submitted to the Ion Torrent Personal Genome Machine®. Single nucleotide polymorphisms (SNPs) were identified using a C++ module and a pileup approach with filters for mapping quality, base quality, read length, and strand bias. In total, 3,898,057 reads from *P. vivax* pools and 1,005,303 reads from sequencing controls were obtained and analyzed for SNPs. Control sequences demonstrated no false-positive SNPs at the quality cut-offs used. In addition, simulations validated the C++ module for SNP detection to 0.5% frequency within each pool. Among the eleven pools, we found 23 SNPs across twelve codons in the kelch propeller region. Twelve of the SNPs produced nonsynonymous mutations, none of which were maintained year-to-year. Two synonymous mutations persisted over multiple time-points. Five mutations were shared between parasite populations, of which, one mutation (V552I) has been previously reported in northeastern Cambodia. However, none of our detected SNPs produced orthologous artemisinin-resistance conferring *P. vivax* mutations and did not persist within the populations, which suggests a lack of directional selection on the K12 propeller region attributable to artemisinin drug pressures. Next-generation sequencing described several SNPs that were not described previously by traditional surveillance techniques, providing a timely way to conduct in-depth molecular surveillance for early artemisinin resistance detection.

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EVOLUTION OF SOLUBLE HLA-G LEVELS DURING PREGNANCY AND INFANCY IN A BENINESE POPULATION EXPOSED TO MALARIA INFECTION

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Human Leucocyte Antigen-G is a non-classical HLA class I molecule firstly described on the surface of extravillous cytotrophoblast cells at foeto-maternal interface. HLA-G binds its inhibitory receptors present on the surface of immune cells (monocytes, NK, T,B and dendritic cells) modulating host's immune response.These immunosuppressive properties of HLA-G are crucial and benefic during pregnancy where HLA-G plays a crucial role in maternal-fetal tolerance. There are known associations between high levels of circulating soluble HLA-G (sHLA-G) and either parasite or viral infections (HIV,cytomegalovirus)and it has been suggested that the induction of sHLA-G expression could be a mechanism via which infectious agents subvert host immune defence. To explore more precisely interactions between soluble HLA-G and malaria,latent class analysis was used to test whether distinct sub-populations of children, each with distinctive soluble HLA-G evolutions may suggest the existence of groups presenting variable malaria susceptibility. This study was conducted in Benin from 2010 to 2013 and 165 children were followed from birth to12 months and soluble HLA-G was quantified by Elisa method.Three groups of children were identified: one with consistently low levels of soluble HLA-G during follow-up, a second with very high levels and a last intermediate group. In all groups, low birth weight, malaria infection and high exposure to malaria transmission were associated with high level of soluble HLA-G.Placental malaria was not. Presence of soluble HLA-G in cord blood increased the probability of belonging to the highest trajectory. These results, together with previous ones,confirm the importante role of HLA-G in the individual susceptibility to malaria. Assaying soluble HLA-G at birth could be a good indicator of newborns more fragile and at risk of infections during childhood.

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MEMORY T CELLS METABOLISM DURING CHRONIC MALARIA INFECTION

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Malaria infection kills up to 0.85 million people each year. The first generation of vaccine does not generate long-lived protection. We have shown, in *Plasmodium chabaudi* infection that CD4 effector memory T cells generated protect from parasitemia and pathology. However, the mechanisms underlying development and maintenance of this long-lived protective memory T cells (Tmem) are not well understood. Recent findings have highlighted the importance of cellular metabolism in Tmem generation. Specifically, fatty acid oxidation (FAO) has been associated with CD8 Tmem development in acute infection. Substrates for FAO in CD8 Tmem are generated through the fatty acid synthesis (FAS) pathway. However, it's not clear whether FAS pathway controls Tmem differentiation or survival. Using transcriptomic analysis, we found upregulation of FAS genes in Tmem compared to effector (Teff) in malaria-specific CD4 T cells. Interestingly, blockade of FAS pathway *in vivo* using TOFA (*Acc1-specific*), impairs Tmem development. To determine when FAS is required for Tmem differentiation, *P. chabaudi*- infected mice were treated with TOFA at the priming or contraction phase of the immune response. Preventing fatty acid synthesis during priming inhibits memory formation and reduces parasitemia. Using stable isotope tracer, memory T cells show high phospholipids synthesis. Together these data suggest that an early shift to FAS is important for CD4 Tmem differentiation and may prove crucial to development of malaria vaccine.

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IL-15 COMPLEX-STIMULATED NK CELLS PROTECT MICE FROM CEREBRAL MALARIA

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To date, no effective adjunctive therapies exist for severe malaria. *Plasmodium falciparum* is the main cause of severe malaria in humans and accounts for about 600,000 deaths per year, mainly in children in sub-Saharan Africa. Cerebral malaria (CM) is one of the most lethal complications of severe malaria. Infection of susceptible mouse strains such as C57BL/6 with *Plasmodium berghei* ANKA (PbA) induces a fatal neurological syndrome from 6-10 days post-infection (dpi). We found that prophylactic or therapeutic treatment of C57BL/6 mice with interleukin (IL)-15 complexes (IL-15C; IL-15 bound to an IL-15Rα-Fc fusion protein) prevented the development of PbA-induced CM. IL-15C treatment stimulates Natural Killer (NK) and CD8 T cells. Interestingly, adoptive transfer of IL-15C-stimulated NK cells, not CD8 T cells, prevented CM. Similar complexes formed with IL-2 (IL-2C; IL-2 bound to the anti-IL-2 S4B6 antibody) also causes robust expansion and activation of NK cells, but NK cells from mice treated with IL-2C failed to protect against CM. Comparative RNAseq analysis of IL-15C and IL-2C-treated NK cells identified novel gene expression patterns, demonstrating previously unappreciated differences between these cytokine complex signaling cascades in NK cells. Interestingly, IL-15C treatment resulted in reduced CD8 T cell activation in the brain at 6 dpi and reduced blood brain barrier breakdown, suggesting that IL-15C-stimulated NK cells limit the CD8 T cell-mediated pathology in CM. Indeed, a large subset of NK cells in the spleen, blood, and brain of IL-15C-treated, but not IL-2C-treated, mice produced the immunoregulatory cytokine IL-10 on day 3 pi. These data indicate that NK cells - which are typically involved in promoting inflammatory responses - can restrain damaging immune responses. A mechanistic understanding of CM pathogenesis and the process of cytokine complex perturbation will provide an important foundation for the identification of new therapeutic targets and aid in the development of adjunctive therapies for treating severe malaria.

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THE ROLE OF INFLAMMATION AND MICROVASCULAR DAMAGE/REPAIR IN THE PATHOGENESIS OF CEREBRAL MALARIA

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Cerebral Malaria (CM), a severe form of malaria, caused by *Plasmodium falciparum* remains a major cause of morbidity and mortality. Currently, there is no available test to predict potential CM patients, as well as mortality or recovery from the syndrome. The disease results from a combination of vascular and inflammatory immune system dysfunction. Triggering receptor expressed on myeloid cells 1 (TREM-1) has been shown to potentiate inflammatory response. A recent preliminary study has

shown that there is an increase in soluble TREM-1 production in CM as compared to uncomplicated malaria (UM). Another study in our lab has shown that there is lower levels of endothelial progenitor cells (EPC) in CM children as compared to UM and Healthy controls (HC). Based on this result, it could be suggested that there could be an association between inflammation and microvascular damage/repair in the pathogenesis of cerebral malaria. To study this hypothesis, children between the ages of 2-12 years who are either CM, UM or HC have been recruited into the study. Samples were taken at three or four time points i.e. Day 0, (Recovery-for only CM), Day 7 and Day14. TREM-1 data and EPC data would be correlated to give a better insight into cerebral malaria pathogenesis. Findings from this study could be employed in the diagnosis of CM.

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CLINICAL DEVELOPMENT OF A VAR2CSA-BASED PLACENTAL MALARIA VACCINE PLACMALVAC: QUANTIFYING VACCINE ANTIGEN-SPECIFIC MEMORY B & T CELL ACTIVITY IN BENINESE PRIMIGRAVIDAE

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Pregnancy associated malaria (PAM) is a major public health problem associated with poor pregnancy outcomes that commonly include maternal anemia and fetal growth alterations, whilst neonatal and infant health can also be affected. A malaria vaccine that targets the pre-erythrocytic stages of the parasite will not prevent the consequences of PAM. The identification of the parasite antigen VAR2CSA that is implicated in the pathophysiology of PAM has led to the development of a candidate vaccine by an EU-funded consortium (PlacMalVac project: German, Danish, French and Beninese Partners). The vaccine is currently under Phase I trial in Germany and Benin. As part of the PlacMalVac project, we quantified B and T cell memory responses to the VAR2CSA sub-unit vaccine candidate in a cohort of pregnant primigravid Beninese who were followed up throughout pregnancy. Clinical and parasitological data were collected every month from 37 primigravid women recruited at the beginning of their pregnancies and followed through to delivery. Mononuclear cells from peripheral blood collected on 4 occasions (first and fifth month of pregnancy, at delivery and 6 months post-delivery) were isolated and cryopreserved under liquid nitrogen. The concentrations of the cytokines IL-5, IL-6, IL-10, IL-13, IFN-γ and TNF-α produced in response to the vaccine antigen, to PPD and to PHA were quantified in supernatants of stimulated cells using cytometric bead array. The frequencies of vaccine antigen-specific antibody-secreting memory B cells were evaluated in the same cell samples using standard ELISPOT assays. Preliminary analysis shows that the profile of vaccine-specific B cell populations increased as a function of women's *Plasmodium falciparum* infection histories, whilst tetanus toxoid-specific B cell frequencies increased following tetanus vaccine boosts administered according to national guidelines. Multivariate analyses are under way to illustrate in detail the effects of *P. falciparum* infections during first pregnancies on the establishment of cellular immunological responses to the vaccine antigen.

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THE INFLUENCE OF INHIBITORY MOLECULES ON TREG CELLS DURING *PLASMODIUM VIVAX* MALARIA

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Malaria is still considered a major helath problem worldwide, and the *Plasmodium vivax* is the most spread causative agent, with 80% of incidence in Brazil. The balance between pro- and anti-inflammatory responses is essential to limit immune response-mediated pathology and regulatory T cells (Treg) probably play an important role in this process. Recently our group demonstrated that the expression of inhibitory receptors on T cells regulates cytokines production by *P. vivax*-specific cells. The expression of one of these inhibitory receptors, the programmed death- 1 (PD-1), negatively regulates Treg function in patients chronically infected with HCV. Since the function of *P. vivax*-specific T cells is impaired due to inhibitory receptors expression, our goal is to assess the expression of these receptors on Treg upon malaria infection and to evaluate their function. Our hypothesis is that the increased expression of inhibitory receptors on Treg during *P. vivax* infection, affects the functions of these cells in regulating inflammatory responses. Peripheral blood mononuclear cells were collected from *P. vivax*–infected patients and from the same individuals after treatment, in Porto Velho-RO. Leukocytes were analyzed by flow cytometry. Our data show that *P. vivax* infection triggers an increase in the frequency of Treg cells and in the frequency of cytotoxic T lymphocyte attenuator (CTLA-4) and PD-1 expressing Treg. The expression of CTLA-4 and PD-1 on Treg was correlated with the bilirubins serum levels. Importantly, PD-1$^+$ Treg express lower levels of Forkhead Box 3 (FoxP3) than PD-1$^-$ Treg when analyzed *ex vivo* or after culture. Moreover, PD-1$^+$ Treg become able to produce IFN-γ. All together, our results indicate that malaria infection triggers the expression of PD-1 and decreases the expression of FoxP3 in Treg, phenomenum that could affect its regulatory functions.

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ANTIBODIES TO *PLASMODIUM FALCIPARUM* APICAL MEMBRANE ANTIGEN-1 AND CIRCUMSPOROZOITE PROTEIN ARE ASSOCIATED WITH PROTECTION FROM HOSPITALIZATION AFTER SEVERE MALARIA DISEASE

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Severe malaria is a leading cause of morbidity and mortality in children. We hypothesize malaria-specific antibodies are markers of exposure and immunity; higher antibody levels will protect children against subsequent hospital sick visits and admission. A prospective cohort study was conducted at Mulago Hospital in Kampala, Uganda. Children between 18 months and 12 years with severe malaria were enrolled: cerebral malaria (CM, n=221), severe malarial anemia (SMA, n=198); and age-matched community controls (CC, n=170) and followed for one year. At enrollment, serum samples were collected and assessed for IgG antibody levels to apical membrane antigen-1 (AMA-1), circumsporozoite protein (CSP), glutamate rich protein (GLURP) and merozoite surface proteins-1 (MSP-1) using a multiplex assay. Children with SMA were the youngest, 33.5 months 41.0 months (CM), and 46.3 months (CC). Children with CM and SMA had significantly higher antibody levels for all antigens. Children with CM had higher antibody levels against malaria-specific antigens than children with SMA or CC, $p<0.05$ for all comparisons. The rate of returning sick visits for clinical malaria in the year following enrollment was 19.5% (n=43) for children with CM, 23.2% (n=46) for children with SMA, and 15.3% n=26 for CC. Higher antibody levels to AMA-1 were associated with protection from clinical malaria in CM and CC, (odds ratio

(OR), 95% confidence interval (CI): CM, 0.97(0.95, 1.00), p=0.05; CC, 0.97(0.95, 1.00), p=0.05). Following adjustment for age, the antibody levels against AMA-1, CSP and MSP-1were not protective against clinical malaria for all comparisons, (OR) 95% (CI) 0.99, (0.92, 1.06), P=0.71; 1.04, (0.98, 1.00), p, 0.17; 0.94, (0.88, 1.00), P=0.06), respectively. Despite having higher exposure to malaria, children with severe malaria were not protected from clinical malaria in the year following admission. These data suggest that acquisition of IgG antibodies against the antigens we measured may not be sufficient to protect against clinical malaria. Further studies are needed to look at IgG subclass responses in this cohort.

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ANTIBODY DEPENDENT CELLULAR INHIBITION IS ASSOCIATED WITH PROTECTION AGAINST FEBRILE MALARIA IN A LONGITUDINAL COHORT STUDY INVOLVING GHANAIAN CHILDREN

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The antibody dependent respiratory burst and opsonic phagocytosis assays have been associated with protection against malaria; however, other mechanisms may also be involved. The antibody dependent cellular inhibition (ADCI) assay is yet to be correlated with protection in longitudinal cohort studies (LCS). We investigated the relationship between ADCI activity of immunoglobulin G prior to malaria season and risk of malaria in a LCS involving 98 Ghanaian children. Purified IgG was tested in ADCI assay and in schizont extract Enzyme-Linked Immunosorbent Assay. Antibody-dependent cellular inhibition activity increases with age and high ADCI (75% SGI) activity was significantly associated with protection against malaria. The importance of IgG3 in the ADCI mechanism was also substantiated. In conclusion, findings here suggest a potential usefulness of the ADCI assay as a correlate of protection to guide malaria vaccine studies.

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THE PRESENCE, PERSISTENCE AND FUNCTIONAL PROPERTIES OF DUFFY BINDING PROTEIN II ANTIBODIES ARE INFLUENCED BY HLA CLASS II ALLELIC VARIATIONS

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Plasmodium vivax infects human reticulocytes through the interaction between the Duffy binding protein (region II, DBPII) and its cognate receptor on reticulocytes, the Duffy antigen/receptor for chemokines (DARC). A high proportion of individuals naturally exposed to P.vivax fails to develop antibodies that inhibit the DBPII-DARC interaction, and genetic factors that modulate humoral immune response are poorly characterized. Here, we investigate if DBPII responsiveness could be HLA class II-linked. A community-based open cohort study was carried-out in a community of the Brazilian Amazon region, in which 336 non-related volunteers were genotyped for HLA class II (DRB1, DQA1 and DQB1 loci), and their DBPII immune responses were followed-up (baseline, 6 and 12 months) by conventional serology (DBPII-IgG antibodies) and functional assays (DBPII-Binding Inhibition Antibodies, BIAbs). In silico analyzes evaluated the relative binding affinities of DBPII peptides for class II molecules associated with distinct immune outcomes. After 12-month follow-up, the results demonstrated an increased susceptibility of DRB1*13:01 carries to develop and sustain their DBPII-IgG antibody response, and strengthen as persistent non-responder individuals harboring the haplotype DRB1*14:02-DQA1*05:03-DQB1*03:01. The HLA class II polymorphisms also influenced the functional proprieties of DBPII antibodies, with three alleles (DRB1*07:01 DQA1*02:01 DQB1*02:02) that comprise a single haplotype linked with the presence and persistence of the BIAbs response. Finally, quantitative prediction of DBPII-HLA class II binding affinity demonstrated that the nonresponse to DBPII was not due to failure of immune epitopes to bind HLA class II molecules. In conclusion, the current study confirms the heritability of DBPII antibody response, with genetic variation on HLA class II influencing in both the development and persistence of IgG antibody responses. Further studies on knowledge of the binding affinities of DBPII peptides for class II molecules linked with antibody responses might be useful for vaccine development.

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UNCOMPLICATED MALARIA CHILDREN AND ADULTS WITH IN MALARIA HYPERENDEMIC AREA OF BURKINA FASO TREATMENT AND ANTIBODIES PRODUCTION

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Artemisinin-based Combination Therapies (ACTs) are the first line drug for the treatment of uncomplicated malaria in most endemic countries. They quickly clear the parasitaemia and reduce fever. In animal model, it has been found that artemisinin derivatives have an immunosuppressive effect. In the present study we assessed the effect of ACT on malaria antigens specific antibodies in population living in malaria hyperendemic area and repeatedly having uncomplicated malaria.In 2013, patients presenting with uncomplicated malaria were recruited and allocated to receive ACTs and follow up to 2 years. Antibodies titer against three P.falciparum blood stage antigens (MSP3, GLURP R0, GLURP R2) were measured by ELISA before and twenty eight days after treatment and during subsequent uncomplicated malaria episodes.In total 478 volunteers were recruited for antibody measurement. Antibody levels were always high Twenty eight days after the initiation of the treatment for all tested antigens but not significative. IgG titer measurement for MSP3 antigen show a trend between D0 and D28 with respectively 7.52 [1.5 13.6] AU and 7.85[2.5 13.1] AU. Subsequent malaria episodes also appear to have a boosting effect on antibody responses.Concomitant parasitaemia initiate and boost immunological responses in population naturally exposed to malaria and Artemisinin-based Combination Therapies seem not to have immunosuppressive effect.

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CELLULAR IMMUNE RESPONSES FOLLOWING CONTROLLED HUMAN MALARIA INFECTIONS BY DIRECT VENOUS INOCULATION OF CRYOPRESERVED *PLASMODIUM FALCIPARUM* SPOROZOITES IN MALARIA-NAÏVE, MALARIA-IMMUNIZED AND SEMI-IMMUNE AFRICAN INDIVIDUALS

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Controlled human malaria infection (CHMI) trials represent an important tool to test vaccine and drug efficacy against *Plasmodium falciparum* malaria, as well as to study the immune response of the human host under controlled conditions. We studied B and T cell responses in volunteers with three different malaria-immunology backgrounds from CHMI trials performed in Europe and Africa. All volunteers received a direct venous inoculation (DVI) of 3,200 cryopreserved *P. falciparum* sporozoites (PfSPZ, Sanaria® PfSPZ Challenge), with cellular studies performed at baseline (prior to challenge), and at different time points following challenge. Trials: 1. Malaria-naïve individuals: Barcelona, Spain; 2. Malaria-immunized individuals: Tübingen, Germany, volunteers were challenged 10 weeks after being immunized with PfSPZ Challenge by DVI or placebo both under chloroquine treatment (Sanaria® PfSPZ-CVac); 3. Lambaréné, Gabon, semi-immune Gabonese volunteers, including both sickle cell trait (AS) and no sickle cell trait (AA) individuals. The study included volunteers who developed patent parasitemia and also those who remained protected following challenge. B cell phenotyping was performed in all samples *ex vivo* by flow cytometry (FACS) with a panel of 11 markers aimed at analysing different B cells subsets including classical, atypical and marginal zone-like memory B cells. T cellular studies were performed after *in vitro* stimulation with NF54 *P. falciparum* infected red blood cells (all samples) and PfSPZ (only a subset of samples), with a cellular panel of 11 markers aimed at analysing T regulatory cells, γδT cells, cytotoxicity markers (CD107a) and cytokine production (IL-1, IL-10, INFγ). These studies provide relevant information on/about the immune response at B and T cell level in individuals with different malaria-exposure and immunology backgrounds that can/may/should guide the study of correlates of protection in malaria-vaccine trials involving naïve and semi-immune subjects.

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HIGH TOTAL IGG LEVELS AND IGG1 SUBCLASS AGAINST MSP10 PROTEIN ARE ASSOCIATED TO PROTECTION IN ASYMPTOMATIC SERA FROM *PLASMODIUM FALCIPARUM* INFECTED PATIENTS FROM THE PERUVIAN AMAZON REGION

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A previous study conducted by our group demonstrated that recombinant protein MSP10 was highly reactive in sera from symptomatic and asymptomatic patients infected with *Plasmodium falciparum* from a low transmission setting in the Peruvian Amazon region. The aim of this study was to evaluated the humoral response in sera from 122 symptomatic (Sym), 21 asymptomatic (Asym) patients infected with *P. falciparum* and 20 controls negative (Ctr) against recombinant MSP10 (rMSP10) and to compare total IgG and subclass profiles (IgG1, IgG2 and IgG3) in naturally exposed individuals living in this region by ELISA. Total IgG responses, calculated as optical density (OD), were significantly higher in Asym vs. Sym and both groups had a higher OD levels compared to Ctrl. Likewise, IgG1 subclass showed a significant difference between the 3 groups with higher OD mean level in Asym followed by Sym. IgG2 showed significant differences between Asym vs. Control and Sym vs. Control. Nevertheless, IgG3 only had basal levels and a significant difference between Asym vs. Ctrl and showed the lowest OD levels. No negative correlation was found between parasitemia and humoral response in any of the groups.The results here demonstrated that rMSP10 was able to induce a differential response in IgG1 levels between Sym and Asym being higher in the last group showing association to clinical protection against this antigen. It was also found that total IgG levels were high in both Asym and Sym patients in comparison to Ctrl confirming our previous results. Interestingly, this preliminary study shows rMSP10 as a potential antigen for the identification of asymptomatic individuals which are known to represent an infectious reservoir in malaria endemic areas. Definitely a larger sample number would be needed to ascertain this.

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IS TIMING OF *IN UTERO* EXPOSURE KEY IN PREVENTING FETAL PRIMING?

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In malaria endemic regions, pregnant women are at increased risk for malaria. Prenatal exposure to malaria parasites or their soluble antigens modifies subsequent immune responses and susceptibility to malaria infection. Timing of *in utero* exposure may be the key in whether immune imprinting occurs. In this study we examined whether the timing of exposure and early detection and treatment of malaria in mothers can prevent or limit fetal priming and the resultant phenotypes. 300 pregnant Kenyan women were enrolled in the study. Mothers received malaria prophylaxis during the antenatal care (ANC) visits and were treated if found positive for malaria during pregnancy. Malaria was diagnosed by blood smear and PCR. We examined T cell immunity to *Plasmodium falciparum* merozoite surface protein-1 (MSP1-42) and the peptides to MSP1-42 in cord blood lymphocytes (CBL) and assayed for lymphocyte proliferation and used Bioplex to detect IL-2, IFN-γ, IL-13, IL-5, IL-10 and TNF-α. Newborns were categorized as: i) malaria +ve at ANC visits and delivery (n=21) ii) malaria +ve at ANC visits and -ve at delivery (n=195) iii) malaria -ve at ANC visits and delivery (n=84). Preliminary analysis was done

using one way ANOVA with Dunnetts multiple comparisons to compare the 3 groups. Data, though not statistically significant, shows a reduced proliferation to malaria-antigens in offspring of women positive for malaria at delivery as compared to the malaria negative. Th2 response and IL-10 was greater in offspring of mothers with malaria at ANC and delivery (mean IL-13 273.9 pg/ml vs 2.86 pg/ml; IL-5 20.56 pg/ml vs 3.09 pg/ml and IL-10 27.5 pg/ml vs 1.03 pg/ml); though data was not statistically significant. These preliminary results show a high cellular immune response to malarial Ags in CBL isolated from neonates whose mothers were exposed to malaria later in pregnancy as compared to those exposed early into pregnancy. Further testing and analysis is ongoing to determine the implications of early detection and treatment of antenatal malaria on neonates' immunity to malaria.

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PHAGOCYTIC FUNCTION OF MONOCYTE SUBSETS DURING ACUTE UNCOMPLICATED MALARIA IN KENYAN CHILDREN

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Monocytes play an important role in innate and adaptive immunity to malaria. Human blood monocytes are classified into 3 subsets according to levels of CD14 and CD16 expression (classical $CD14^{hi}CD16^{-}$, intermediate $CD14^{hi}CD16^{+}$, and nonclassical $CD14^{lo}CD16^{+}$). The functional roles of the subsets during malaria infection are still being described. Cryopreserved peripheral blood mononuclear cells (PBMC) were obtained from 9 children in western Kenya at presentation with acute uncomplicated malaria and 6 weeks following treatment. Phagocytic activity was determined using assays in which CFSE-labeled Pf-infected erythrocytes (IE) were opsonized with heat-inactivated plasma (pooled Kenyan adult (KA) plasma as a positive control, or malaria-naïve North American (NAM) plasma as a negative control). After incubation of IE with PBMC, the cells were stained with fluorescently-labeled anti-CD14 and anti-CD16 antibodies and subjected to flow cytometry. The percentage of monocytes within a population that had phagocytosed IE was calculated according the the CFSE signal. Opsonic phagocytic activity of classical monocytes and all monocyte subsets combined was decreased during acute malaria compared to 6 week recovery (classical subset median values 20.8% vs. 28.9%, $p < 0.01$; all monocytes 25.5% vs. 43.8%, $p < 0.01$). Phagocytic activity of the monocyte subsets in the presence of NAM-opsonized IE did not differ between acute and recovery. Intermediate and nonclassical monocytes displayed greater phagocytic activity compared to classical monocytes in the presence of NAM-opsonized IE (9.2% and 7.3% vs. 4.5%, respectively, p values < 0.01) as well as KA-opsonized IE (52.4% and 62.6% vs. 27.8%, respectively, p values < 0.01). These data indicate that opsonic phagocytic function of monocytes is decreased during acute malaria compared to 6 weeks following treatment. Compared to the classical subset, intermediate and nonclassical monocytes were more efficient at phagocytosing IE.

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CD68 REGULATES PARASITE DENSITY OF *PLASMODIUM YOELII* 17XNL MURINE MALARIA

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CD68 is a highly glycosylated transmembrane protein found on macrophages and other immune cells. Although CD68 is a well-known marker for macrophages, its function is poorly understood. Recently, this molecule has been identified as a candidate receptor for *Plasmodium* sporozoite invasion of Kupffer cells– the specialized macrophages that line the sinusoids in the liver. In this study, we measured the effect of CD68 on the erythrocytic stage of *Plasmodium* murine malarias using mice deficient for the *CD68* gene. Loss of CD68 had no effect on susceptibility to experimental cerebral malaria or on parasite burden during lethal *Plasmodium berghei* ANKA infection. However, absence of CD68 resulted in a two-fold increase in parasite density at peak parasitemia (day 10 post-infection) during non-lethal *P. yoelii* 17XNL (*Py* 17XNL) infection. To better understand the immune mechanism of CD68-mediated control of *Py* 17XNL parasitemia, we compared the major immune cell subsets by flow cytometry and measured serum cytokine and antibody levels in wildtype verses mice lacking the *CD68* gene over the course of *Py* 17XNL infection. Significant differences were observed in the number of macrophages, natural killer cells, and B cells expanded in the spleen. In addition, production of the IL-12p70, IL-10, MCP1, MIP1β, G-CSF, and RANTES cytokines were significantly altered in the absence of CD68. Lastly, there was a dramatic difference in the quantity of IgG in the serum of WT verses CD68 knockout mice, suggesting that CD68 may alter the B cell response during *Py* 17XNL malaria. Studies are underway to determine the role of CD68 in the differentiation of macrophages into the M1 and M2 subsets, and to dissect the mechanism of CD68-mediated regulation of B cell immunity by *in vivo* depletion experiments. The results of these studies will be useful in discerning the function of CD68 and in understanding the requirements for immune protection to malaria.

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TACI CONTRIBUTES TO *PLASMODIUM YOELII* HOST RESISTANCE BY CONTROLLING THE KINETICS OF TFH AND GC FORMATION AND ANTIBODY DEVELOPMENT

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The transmembrane activator and calcium-modulator and cyclophilin ligand receptor (TACI) is involved in B-cell survival, antibody isotype switching and plasma cell generation. TACI expression is severely impaired in murine and human newborns as compared to adults. We challenged TACI knock-out (KO) mice with *P. yoelii* (Py) NL in order to evaluate the role of TACI in response to malaria infection. We found that the parasitemia levels were significantly elevated in TACI KO-mice (61.8% at day 18) compared to C57BL-6 WT-mice (11.1% at day 11), and that parasite clearance was substantially delayed in the TACI KO (27 days) mice relative to WT controls (18 days). We also determined that TACI KO-mice have a delay in anti-Py NL IgG-antibody production when compared with the WT-mice. Since the interaction of T follicular helper (Tfh) cells with antigen specific B cells in the germinal center (GC) is essential for the generation of antibody responses against T cell-dependent antigens, we measured the formation of Tfh and GC in the spleens of Py NL infected mice. Interestingly, we determined that while Tfh cell numbers were elevated on day 10 in both the strains, WT mice Tfh cell numbers sharply declined by day 15 as the number of Tfh cells in TACI KO-mice remained high. Coinciding with parasite clearance kinetics, TACI KO Tfh cells declined to baseline values on day 25. Similar to the Tfh cells, GC B cells remained high in TACI KO-mice after their decline on day 15 in WT mice. Finally, we detected elevated serum levels of the TACI ligand B cell-activating factor belonging to the TNF family (BAFF) in infected TACI KO mice as compared to the WT mice. Conclusion: Susceptibility of TACI deficient mice to Py NL infection appears to be based on altered Tfh and GC kinetics, which in turn results in delayed antibody development.

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IMMUNOLOGICAL EFFECT OF SEASONAL MALARIA CHEMOPREVENTION (SMC) WITH SULFADOXINE-PYRIMETHAMINE (SP) AND AMODIAQUINE (AQ) IN CHILDREN UNDER 10 YEARS IN THE SOUTHEASTERN PART OF SENEGAL

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In developing countries, malaria is still a major public health problem and children are the most affected individuals. In order to strengthen malaria control, Seasonal Malaria Chemoprevention (SMC) has been developed. This strategy is very effective in preventing malaria clinical episodes but its effect on children's immunity is not well documented. This study aimed to assess the immunological effects of SMC among children under 10 years living in the southern part of Senegal (Velingara). The study was nested in a cluster randomized trial assessing the impact of SMC with a single dose of Sulphadoxine-Pyrimethamine (SP) and 3 doses of Amodiaquine (AQ). Two cross-sectional surveys were carried out at baseline (October 2010) and a year after intervention (September 2011). Thick and thin blood smears were used to assess malaria prevalence. Blood was collected on filter paper for serological measurement by ELISA to measure IgG anti-MSP1_42 and anti-AMA1. Logistic regression analysis was performed to assess factors associated with the production of antibodies. A total number of 1611 children under 10 were included (866 children in 2010 and 745 children in 2011). Malaria prevalence was 10.39% in 2010 and 5.03% in 2011. The seroprevalence of anti-MSP1_42 anti-AMA1 antibodies was higher in 2010 compared to 2011 providing a significant reduction of IgG production at 11.4 AU (95%CI [8.3-14.4]) for MSP1_42 and 7.2 AU (95%CI [4.5-9.9]) for AMA1. Seroprevalence increased with age and *Plasmodium falciparum* carriage while it decreased according to the area and the study period. In conclusion, SMC is an effective strategy for malaria prevention in children under 10 years. The strategy can as well induce a decrease of IgG anti-AMA1 and anti-MSP1_42 which are associated with the protectiion against malaria. Consequently this strategy needs to be renewed each year in areas where malaria is highly seasonal to avoid a resurgence of malaria, while promoting the use of other antimalarial interventions.

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MALARIA INTERVENTION SCALE-UP IN AFRICA: STATISTICAL EFFECTIVENESS PREDICTIONS FOR HEALTH PROGRAM PLANNING TOOLS, BASED ON DYNAMIC TRANSMISSION MODELLING

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Malaria prevention and treatment needs to expand, and national strategies and budget allocations be evidence-based. We statistically summarized dynamically simulated relations between intervention coverage scale-up and impact, to inform a malaria module in the Spectrum program planning tool. The dynamic transmission model OpenMalaria was used to simulate health impacts of insecticide-treated net usage (ITNs), indoor residual spraying (IRS), management of uncomplicated malaria cases (CM) and seasonal malaria chemoprophylaxis (SMC) over a 10-year horizon, for a range of African settings with stable endemic falciparum malaria. ITN effectiveness was parameterized by fitting to estimates from Cochrane review of ITN trials. Generalized linear regression models (GLMs) were used to summarize impact patterns in the simulations. GLMs explained 94-97% of variation in simulated post-intervention parasite prevalence (three age groups, three 3-year horizons); 86-97% for case incidence and 74-95% for malaria mortality, which was most stochastic. For a given effective population coverages, CM and ITNs were predicted to avert most infections, cases and deaths, with lower impacts for IRS. Impact of SMC was limited to young children reached. Proportional impacts were similar across ages, larger at lower endemicity, and (except for SMC) largest in low-endemic settings with low seasonality. Vector control and CM, by reducing endemicity and immunity, entailed a partial rebound in malaria mortality among over 5 year olds from around 7 years after scale-up in low-endemic settings. SMC did not reduce endemicity, but slightly shifted malaria to older ages by reducing immunity in children reached. Incremental health impacts for single-intervention scale-up started to diminish noticeably above 40% coverage, while in high-endemic settings CM and ITNs acted synergistically by lowering endemicity. Statistical models can emulate epidemiological dynamics and inform strategic planning and target setting for malaria control.

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UNDERSTANDING THE MECHANISM OF *PLASMODIUM VIVAX* HYPNOZOITES REACTIVATION

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Plasmodium vivax hypnozoites serve as the major source of maintaining *P. vivax* transmission in endemic countries. Controlling this parasite requires a thorough understanding of the factors determining the timing of *P. vivax* hypnozoite reactivation, which is not completely understood. In this study, we analyze previously published data from infected patients who were treated for blood stage infection and subsequently followed-up until detection of *P. vivax* blood stage parasites. We develop a mathematical model of the frequency of hypnozoite reactivation from the liver, and apply this to published treatment-to-infection studies to investigate the frequency of hypnozoite reactivation over time. We first investigate whether the timing hypnoziote reactivation is constant with time or whether there is evidence that reactivation is induced by previous infection, and subsequently slows with time. We fitted our model to the dynamics of *P. vivax* reactivation of military personnel returning from endemic region, and found that reactivation dynamics were consistent with being induced by previous episodes of infection, and then the reactivation rate slowing over time with a half-life of around 60 days (CI: 45- 89). We also analysed time-to-*P. vivax* infection in patients under continual exposure in an endemic area of PNG, and found that although the initial rate of infection was highest in patients infected with *P. falciparum* at baseline, follow by those infected with *P vivax* and those uninfected at baseline, there was evidence for slowing of *P. vivax* reactivation rate with time. Mathematical modeling of the reactivation of *P. vivax* hypnozoites after anti-malarial treatment provides insights into the timing of reactivation, and has important implications for understanding treatment trials and planning eradication strategies.

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USING CELL PHONE DATA TO IMPROVE MALARIA TARGETING AND MITIGATE THE NEGATIVE EXTERNALITY OF INTERNAL POPULATION MOVEMENT

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The effect of population movement on malaria prevalence has been studied through small case control studies or using modeled parasite prevalence maps linked to human movement data. Yet, case control studies only cover a small geographic area and are not representative of a whole region or country, and using parasite prevalence becomes more difficult in areas that are at a pre-elimination stage. In those areas of low transmission, population movement is critical because most new cases

are imported from abroad or other parts of the country, and contribute to secondary cases. This study utilizes a unique dataset of cell phone usage for 9 million people in Senegal to measure population movement, and combines it with monthly malaria case data available at the health post level for the North of the country. This type of analysis, linking detailed movement data from cell phone records to case data on malaria makes it possible to study the effect of population movement in low transmission settings. In addition, movement is broken down into residents returning after travel away from home and visitors coming in from outside areas, in order to better understand which travelers are at the highest risk for spreading the disease. An important effect of population movement on malaria cases is found, especially for the very low malaria transmission districts that receive many travelers. The majority of the effect comes from residents travelling and returning, with each expected infected case coming into an area in the north leading to 2.5 new cases of malaria in that area, and very little impact from visitors. In addition, simulations demonstrate which are the districts in Senegal that are the leading exporters of the disease to low malaria areas of the country, and how targeting these districts could lead to larger decreases in prevalence. This paper also contributes to the research field of malaria more generally, demonstrating how as areas approach elimination, environmental factors like rainfall decrease in importance and human factors, such as travel, play a more prominent role in driving transmission.

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CLUSTER-LEVEL DETERMINANTS OF REACTIVE CASE DETECTION PERFORMANCE IN MALARIA ENDEMIC SETTINGS: A MONTE-CARLO SIMULATION AND MODEL EMULATION STUDY

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In recent years, many countries and regions within countries have achieved unprecedentedly low levels of malaria and are now looking towards elimination. Paradoxically, many current surveillance systems face the challenge of identifying and responding to increasingly rare and often hidden infections. Health officials within these settings are exploring novel approaches to malaria surveillance, such as reactive case detection (RCD), to combat this challenge. Little research has been done to evaluate if such an approach improves the sensitivity of malaria surveillance systems. The aim of this study was to develop a model emulator which predicted the cluster-level sensitivity of RCD, given a set of cluster-level attributes. RCD sensitivity, defined as the proportion of the malaria reservoir detected, was simulated within clusters of geo-coded census data from southern Zambia using a previously described monte-carlo algorithm. Eight rounds of parasite census data were used to maximize variability in the cluster-level attributes, consequently increasing the performance of the model emulator. Cluster-level attributes such as prevalence, treatment-seeking behavior, population density, rainfall, forestation, and others were taken both from the Zambia data and from remote sensing data sources. Program modifiable attributes, such as CHW coverage, were also included in the model emulation. The model emulator was formed by regressing monte-carlo-simulated RCD sensitivity on a function of the cluster-level attributes, including complex interactions between them. Preliminary results suggest that local treatment-seeking behavior and prevalence will be major determinants of RCD sensitivity. The results of this study supplement the limited empirical research on malaria RCD and provide new information for decision making around RCD implementation.

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WITHIN-HOST DYNAMICS AND SPREAD OF DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM* MALARIA

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In the malaria parasite *Plasmodium falciparum*, drug resistance emerges more readily in low-transmission areas than in high-transmission settings. One possible explanation for this phenomenon is that within-host dynamics of *P. falciparum* infections make it harder for drug-resistant parasites to spread in high—transmission settings. With intense transmission, multi-strain infections are common; therefore, a drug-resistant mutant will likely have to compete against several drug-sensitive strains in any host it infects, reducing its chances of survival and onward transmission. To examine the effects of within-host competition on the emergence of resistance, we constructed a nested mathematical model which describes the within-host dynamics of individual infections as well as the dynamics of transmission in the host population. We used the model to examine the effect of transmission intensity on the evolutionary emergence of drug resistance; we also investigated how the impact of transmission intensity depended on host immunity and antimalarial drug use.

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IS THE USE OF HRP2-DETECTING RAPID DIAGNOSTIC TESTS SUFFICIENT TO SELECT FOR HRP2-NEGATIVE *PLASMODIUM FALCIPARUM* PARASITES?

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Successful control and elimination of malaria relies on rapid and accurate diagnosis of clinical cases and surveillance. The use of malaria rapid diagnostic tests (RDTs) has significantly expanded over the past decade with the majority of tests detecting *histidine-rich protein 2* (HRP2), expressed only by *Plasmodium falciparum* (Pf). However, HRP2-negative parasites have been reported in several regions, most notably the South America Amazon, but more recently countries such as India, Myanmar, Ghana, Mali and Senegal. A high prevalence of HRP2-negative parasites can undermine the utility of HRP2-detecting RDTs. While the selection forces on these parasites are unclear it has been hypothesized that the use of HRP2-detecting RDTs could be the primary mechanism for selection of HRP2-negative parasites. In this study we test this hypothesis using an individual-based mathematical simulation model of *P. falciparum* transmission. The purpose was to determine whether diagnosis using HRP2-detecting RDTs alone provides a sufficient selective force to allow newly introduced HRP2-negative parasites to become established within a community, and the probability of this occurring. Results indicate that the probability of successful introduction of HRP2-negative parasites is influenced by transmission intensity, half-life of the ACT companion drug and type of RDT used for diagnosis (HRP2 only vs HRP2/pan-pLDH combination test). In certain circumstances there is also a considerable lag between introduction and clinical evidence of HRP2-negative parasites. Since wide-spread emergence of HRP2-negative parasites has the potential to impact on timely and accurate diagnosis of malaria, improved understanding of the factors facilitating the establishment of non-HRP2 expressing parasites allows for targeted surveillance and monitoring, and subsequently the adaptation of case management strategies in regions with or susceptible to HRP2-negative parasites.

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PLASMODIUM VIVAX AND *P. FALCIPARUM* INFECTION DYNAMICS IN CO-ENDEMIC SETTINGS: RELAPSES, RECRUDESCENCES, RE-INFECTIONS AND THE ROLE OF CO-INFECTION

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Plasmodium vivax infected populations are often co-endemic with *P. falciparum* malaria, with co-infection frequently observed in individuals. *P. vivax* infection dynamics are qualitatively different from *P. falciparum* due to liver-stage infection with hypnozoites which activate to cause relapses. Intensely sampled longitudinal data on genotyped malaria infections is assembled from cohorts spanning the *P. vivax* and *P. falciparum* endemic world, including Papua New Guinea, Solomon Islands, Thailand and Brazil. Individual and population-level infection dynamics are analysed using a mathematical model with statistical inference implemented in a Bayesian framework. Examples are presented where *P. vivax* recurrences are probabilistically classified into relapses, recrudescences and re-infections, depending on primaquine treatment, transmission intensity, and genotype detectability. *P. vivax* infections have shorter durations than *P. falciparum* infections, with the duration of both decreasing in higher transmission settings. We also present multi-site analyses of the association between *P. falciparum* induced fevers and *P. vivax* relapses, and of the impact of co-infection on the duration of blood-stage infections. The combination of longitudinal data, genotyping of samples and statistical analysis presented here allows for probabilistic identification of *P. vivax* relapses and estimation of their contribution to onwards transmission.

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OPTIMIZING THE GLOBAL ALLOCATION OF MALARIA FUNDS

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The burden of *Plasmodium falciparum* malaria remains high and efforts at control are resource-constrained. Optimal allocation of both internal and global financing is therefore paramount. In light of this, and coinciding with the Global Fund's fifth replenishment call we undertook work to inform the allocation and spending of domestic, bi-lateral and multi-lateral funding for malaria control. We used a well-established mathematical model of malaria to describe the cost and impact of varying coverage levels for 4 key interventions (LLINs, IRS, SMC and treatment) across a wide range of epidemiological strata. We used these simulations to estimate the impact of intervention packages on malaria transmission in Global Fund supported countries. We optimised, at the first administrative unit, the spending of domestic financing within country and the distribution and spending of external and Global Fund financing across countries to maximise the number of cases or deaths averted. Targeting interventions can potentially lead to improved impact, with substantial benefits from countries optimising internally. We demonstrate the trade-off between maximising burden reductions and targeting countries for elimination. The optimal allocation is driven by four key factors: baseline transmission intensity, population at risk, current transmission and domestic financing capability.

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DEVELOPMENT OF A NEW SOFTWARE TOOL AND ANALYSIS METHOD TO IMPROVE DETERMINATION OF GLUCOSE-6-PHOSPHATE-DEHYDROGENASE (G6PD) STATUS

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For the radical cure of *Plasmodium vivax* infection, safe administration of 8-aminoquinoline drugs is critical, but these drugs can be administered only after the determination of G6PD status. Cytochemical staining allows for the determination of G6PD activity in an individual red blood cell. It is possible to estimate the relative proportions of G6PD-deficient cells to those with normal G6PD activity. This research describes a software tool to standardize the analysis of flow cytometry in screening for G6PD-heterozygous females. Its primary function is to provide standardized ratios of "normal" to "G6PD-deficient" cells in an individual blood sample. The goal is to determine zygosity of individuals spanning the G6PD polymorphisms found in African and Southeast Asian populations referenced to DNA sequencing methods. Methods: A reference quantitative assay and cytofluorometry method were used to determine G6PD status. A software tool for standardization and interpretation of cytochemical staining for G6PD status has been developed. Criteria for calling zygosity in females was determined using DNA sequencing as the reference assay. The tool was validated with 472 females and males spanning the G6PD polymorphisms found in African and Southeast Asian populations referenced to DNA sequencing methods. Validation of the analysis tool showed 100% sensitivity and 100% specificity for both normal and G6PD-deficient males when referenced to DNA sequencing. For females with varying levels of G6PD activity, the specificity and sensitivity were 91% and 98% respectively for homozygous deficient, 97% and 91% for heterozygous, and 91% and 100% for homozygous normal. In onclusion, a methodology for standardizing the analysis of cytofluorometry in screening for G6PD-heterozygous females has been developed and packaged into a free, open-source software tool that is available online. The software tool is able to correctly call zygosity with a high level of sensitivity and specificity, even across polymorphisms found in Southeast Asian and African populations, making it a useful tool in the identification of G6PD-heterozygous females.

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SPATIAL MODELING AND HETEROGENEOUS ANALYSIS OF THE EFFICACY OF LONG-LASTING MICROBIAL LARVICIDING ON MALARIA OUTDOOR TRANSMISSION IN WESTERN KENYA HIGHLANDS

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The rising insecticide resistance and increase in outdoor transmission have greatly hampered the effectiveness of currently available first line malaria prevention tools such as long lasting insecticidal nets (LLIN) and indoor residual spray methods. Among the alternative intervention methods are being developed and tested in the field, microbial larvicides represent a promising supplemental intervention tool that may tackle outdoor transmission and pyrethroid insecticide resistance. Long-lasting microbial larvicides (LLML) are particularly attractive because reduce the number of reapplications and thus costs associated with insecticide application, and subsequently they may be potentially more cost-effective. To determine the efficacy of LLML in reducing malaria transmission and optimal field application strategies, the Epidemiological Modeling (EMOD) model for Malaria Transmission developed by Institute for Disease Modeling (IDM) was employed to simulate sites with different malaria prevalence and

landscape scenarios through its Computation Modeling Platform Services. Three sites in western Kenya highland and lowland with well characterized vector ecology and malaria prevalence were simulated using the multi-node spatial model with vector migration method of EMOD. The results show that the beneficial killing efficacy of nets might gradually be reduced due to increased insecticide resistance and outdoor transmission in the absence of other supplemental interventions. The potential reduction of population infected rate after LLML application can reach up to 24%. Re-treatment of aquatic habitats every 4 months would lead to consistent reduction in malaria transmission. In summary, the modeling analyses suggest that LLML has the potential to provide significant added benefits to LLIN for malaria control in a range of sites with different transmission intensities and landscape characteristics.

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USING A BAYESIAN GEOSTATISTICAL MODEL TO UNDERSTAND LOCAL-SCALE HETEROGENEITY IN MALARIA RISK: THE EXAMPLE OF BUNKPURUGU-YUNYOO DISTRICT IN NORTHERN GHANA

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Bayesian methods have been used to generate country-level and global maps of malaria risk on a large geographical scale. However, these maps may lack the ability to identify smaller scale heterogeneity and may not be ideal for operational malaria activities. The aim of this study is to apply Bayesian geostatistical models to high-resolution malaria data in order to construct a predictive model of local-scale spatial heterogeneity. We used existing malaria parasitemia survey data from a 30x40km study area in the Bunkpurugu-Yunyoo district of northern Ghana, consisting of 10,366 children from 438 geo-coded communities sampled between November 2010 and November 2013 bi-annually. A Bayesian hierarchical model using a Gibbs sampler and Metropolis Hasting algorithm estimated parameter values for geostatistical predictions and accounted for spatial dependency at individual and community level. To permit generalizability of the model to other districts, we selected only remote-sensed variables, including environmental factors - such as elevation, temperature, rainfall - and GIS-derived demographic factors such as distance to health facility, urban centres, roads and water bodies. Overall, malaria prevalence in the district varied between 19% and 90%, showing a north-east to south-west gradient of predicted risk with the highest prevalence rates found at lower elevations. Model selection revealed elevation and distance to urban centre to be important covariates. The general distribution is heavily weighted between the two modest urban centres, showing lower risk in urban centres compared to rural areas, with some indication that a threshold distance-to-urban-centre exists for malaria risk. Model predictions revealed high variability in malaria prevalence in areas previously assumed to be homogenous, indicating important shortcomings of country level spatial modelling from a programmatic perspective. Our modelling approach could potentially be applied to infer fine-scale malaria risk in other areas of northern Ghana and beyond, using readily available remote-sensed data.

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GEOGRAPHIC TARGETING OF MALARIA INTERVENTIONS IN MYANMAR USING A DYNAMIC ECONOMIC EPIDEMIOLOGICAL MODEL

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Two interventions receive the majority of malaria control funding in Myanmar i) insecticide treated bed nets and ii) early diagnosis and treatment through malaria community health workers. While malaria funding has increased markedly in recent years, universal coverage of both interventions is not currently affordable nor likely to be financially sustainable. This study focuses on 52 priority townships and aims to provide practical recommendations for targeted geographic allocation of these interventions such that impact on malaria is maximised from the investment. Malaria surveillance in Myanmar is undergoing substantial improvement but does not currently capture detailed incidence data from non-governmental organizations. A data repository was established to collect and process historical incidence data from governmental and non-governmental sources. This information was used within a dynamic economic epidemiological model to estimate intervention costs and effects in terms of Disability Adjusted Life Years averted within each township. Township specific intervention recommendations given a fixed total budget are obtained via a resource allocation algorithm. Scenario analysis was employed to illustrate alternative allocation results under variations of certain model or parameter assumptions, such as cost sharing with other disease funds. Finally, uncertainty analysis presents the consistency of intervention allocation result separately for each townships, given the total prior parameter uncertainty. Final results will be completed prior to the conference.

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SIMULATING WITHIN-VECTOR GENERATION OF MALARIA PARASITE DIVERSITY

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Plasmodium falciparum, the most virulent malaria parasite causing disease in humans, undergoes asexual reproduction within the human host, and sexual reproduction within the vector host, *Anopheles* mosquitoes. Consequently, the mosquito stage of the parasite life cycle provides an opportunity to create novel parasite genotypes in superinfected mosquitoes, a likely contributor to the observed high degree of parasite diversity in both high and low transmission settings. This diversity has important implications for disease transmission and malaria control, however the mechanisms driving this diversity within the vector remain under investigation. To understand the role that vector biology plays in modulating the generation of parasite diversity, we developed a two-stage model framework that estimates the genetic diversity across a population of mosquitoes as a consequence of different bottlenecks and expansion events occurring during the vector-stage of the parasite life cycle. In the first stage of this framework, we developed the first stochastic model of within-vector *P. falciparum* parasite dynamics and simulate the dynamics of two competing parasite genotypes, emulating superinfection. Coupled to this model of parasite dynamics is the second stage of our framework: a model of sequence diversity generation through recombination between genotypes within a mosquito. Our model framework demonstrates that bottlenecks from the ookinete to oocyst stage decrease diversity from the initial gametocyte population in a mosquito's blood meal, and increases with the development of sporozoites. Furthermore, the bottlenecks in the transition from the human host to a mosquito population results in a pool of parasites that has increased in diversity at the population level.

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REMOTELY SENSED ENVIRONMENTAL CONDITIONS AND MALARIA MORTALITY IN THREE MALARIA ENDEMIC REGIONS IN WESTERN KENYA

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Malaria is an important cause of morbidity and mortality in endemic countries. The malaria mosquito vectors depend on environmental conditions, such as temperature and rainfall, for reproduction and survival. To investigate the potential for weather driven early warning systems to prevent disease occurrence, the disease relationship to weather conditions needs to be carefully investigated. Where meteorological observations are scarce, satellite derived products provide new opportunities to study the disease patterns depending on remotely sensed variables. In this study, we explored the lagged association of Normalized Difference Vegetation Index (NVDI), day Land Surface Temperature (LST) and precipitation on malaria mortality in three areas in Western Kenya. The lagged effect of each environmental variable on weekly malaria mortality was modeled using a Distributed Lag Non Linear Modeling approach. For each variable we constructed a natural spline basis with 3 degrees of freedom for both the lag dimension and the variable. Lag periods up to 12 weeks were considered. The effect of day LST varied between the areas with longer lags. In all three areas, malaria mortality was associated with precipitation. The risk increased with increasing weekly total precipitation above 20 mm and peaking at 80 mm. The NDVI threshold for increased mortality risk was between 0.3 and 0.4 at shorter lags. This study identified lag patterns and association of remote-sensing environmental factors and malaria mortality in three malaria endemic areas in Western Kenya. Our results show that rainfall has the most consistent predictive pattern to malaria transmission in the study area. Results highlight the potential for developing locally based early warning forecasts that could reduce the disease burden by enabling timely control actions.

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EFFECT OF IMMEDIATE VS. DELAYED IRON THERAPY ON NEUROCOGNITIVE OUTCOMES IN CHILDREN WITH SEVERE MALARIA

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Malaria and iron deficiency are associated with neuropsychological deficits, but iron supplementation given concurrently with antimalarial treatment per the standard of care may not be well absorbed due to malaria-induced inflammation. We compared neuropsychological functioning after 6 and 12-months between children with cerebral malaria (CM) or severe malarial anemia (SMA) who received ferrous sulphate on admission concurrently with antimalarial treatment (immediate group) or four weeks after admission (delayed group). We hypothesized that children with delayed iron treatment would have better iron uptake, therefore better neurocognitive outcomes at 12-months follow-up. We recruited 239 Ugandan children aged 18 months - 4.9 years for 12 months: 79 with CM, 77 with SMA, and 83 healthy community children (CC). All CM, SMA and 35 CC were iron-deficient (zinc protoporphyrin (ZPP)≥80 mmol/mol heme) and randomly assigned to start a 3-month course of ferrous sulphate immediately or after four weeks. Children were assessed for overall cognitive ability, attention, and associative memory 1 week and 6 and 12 months after admission. Administration of iron immediately vs. delayed did not lead to significantly different neurocognitive outcomes in cognitive ability, attention or associative memory in children with CM or SMA at 12-months follow-up. However, a mixed effects model analysis that included outcomes at all three time points showed that children with CM in the immediate treatment group exhibited better attention scores than the delayed group. Regardless of treatment arm, CM had significantly worse cognitive ability and associative memory scores, and SMA had significantly worse cognitive ability scores compared to CCs at 12-months. Delay of iron may not result in improved long-term neurocognitive outcomes in children with severe malaria as compared to immediate iron treatment. In children with CM, it was associated with worsened long-term attention. Administration of iron therapy with either timing may not prevent worsened neurocognitive outcomes in children with severe malaria when compared to community children in the area.

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IMPROVING REPORTING OF WEEKLY MALARIA DATA THROUGH THE ELECTRONIC INTEGRATED DISEASES SURVEILLANCE AND RESPONSE (E-IDSR) IN TEN REGIONS OF TANZANIA

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To ensure that malaria epidemics are detected and addressed within two weeks of onset, the Tanzania National Malaria Control Program monitors malaria cases reported weekly via the electronic Integrated Disease Surveillance and Response System (e-IDSR). Implementation of the e-IDSR started with 67 health facilities (HF) in November 2013 and progressively scaled-up to reach 2,967 HFs by January, 2016, covering a total of 10 out of 25 regions. This study reports trends in reporting rate, gaps, and the ongoing initiative to improve low reporting rates. At the HF a health worker compiles data for each epidemiological week (Monday to Sunday) and submits the previous week's data via mobile phone by dialing number *152* 05#. Reports are submitted every Monday by 3:30pm, later submission is considered late reporting. Malaria data reported are number tested with RDT/microscope, positive and clinical malaria case. The submitted report goes directly into the DHIS2 information management system and can be accessed by officials at district, regional, and national levels. Overall, in 2013 the reporting rate was 49%, 63.3% in 2014, and 38.3% in 2015. Reports received on time (by Monday 3:30 pm) were 35.6% in 2013, 36.4% in 2014 and 10.8% in 2015. There was considerable variation in reporting rate between districts. In 2015, only 3(5%) out of 60 districts had reporting rates which met the national target of 80% (range 80%-84%). 13(22%) out of 60 districts had a reporting rate ranging from 50%-74%. Overall malaria data reported in 2013 showed that a total of 54,985 people tested of which 15, 654 (28%) had malaria, in 2014 and 2015 a total of 1,211,789(87%) and 2, 458,807 (89%) people were tested of which 474,556(39%) and 817,494(33%) tested positive for malaria respectively. Complete and timely reporting of malaria cases is crucial to prevent and mitigate malaria outbreaks. The e-IDSR reporting rate remains far below target and has faltered after three years. To improve reporting a series of 3 day workshops incorporating practices and lesson learned from the best performing districts is being conducted and attended by regional and district authorities in all 10 regions.

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"I FEEL SO BAD BUT HAVE NOTHING TO DO." A QUALITATIVE STUDY OF UGANDAN CAREGIVERS' EXPERIENCES OF PARENTING WITH CHILDREN WITH SEVERE MALARIA AND SUBSEQUENT REPEATED EPISODES OF UNCOMPLICATED MALARIA

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Severe malaria (SM) and repeated malaria attacks (RMA) are major public health concern. In the endemic regions, children experience 2-5 malaria attacks annually. The experience of caregivers with children having repeated malaria attacks has not been documented. The purpose of this study was to explore caregivers' experiences in parenting of a child with a history of severe malaria followed by repeated episodes of uncomplicated malaria. This was a qualitative study that used phenomenological approaches. Twenty five caregivers of children previously exposed to severe malaria and who had experienced repeated subsequent episodes of uncomplicated malaria were purposively selected. Data was collected using in-depth interviews conducted in Luganda, a native dialect. Interviews were audio recorded, transcribed and translated into English language. Data was manually analyzed using content analysis. The main themes and subthemes exploring caregivers' experiences of parenting a child with a history of severe malaria and repeated malaria episodes were generated. From the interviews, the 4 main themes were identified. These included; societal burden where children are left with community members when their caregivers have to inevitably work; disagreements in seeking healthcare from traditional, spiritual or modern medicine among caregivers, family and the community; family life disruptions involving breakdown of relationships and inadequate male-spouses involvement in the child care; and a sense of helplessness in management of a child with severe malaria and repeated malaria episodes. Severe malaria and repeated malaria episodes affect not only the children who have these illnesses but also their caregivers' parenting experiences. There is need for caregiver-parenting sessions towards the management of children who have had severe malaria and repeated subsequent episodes of malaria.

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PREVALENCE OF SEVERE VIVAX MALARIA: A SYSTEMATIC REVIEW AND META-ANALYSIS SINCE 1900

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Malaria caused by *Plasmodium vivax* was long considered to have a low mortality, but recent reports suggest that severe and complicated vivax malaria may be more common than previously thought. The primary objective of this systematic review and meta-analysis was to describe the reported clinical characteristics and the geographical variation in prevalence of reported severe vivax malaria and its change over time derived from English-language articles published since 1900. Medline and Scopus databases were searched for original papers on severe vivax malaria. A total of 77 studies with reported severe vivax malaria and 63 studies with no reported severe vivax malaria (totaling 46,411 and 6,753 vivax malaria patients, respectively) were included. The 77 studies with reported severe vivax malaria were mainly from India (n = 33), USA (n = 8), Indonesia (n = 6), and Pakistan (n = 6). Among the 77 studies reporting severe vivax malaria, severe thrombocytopenia (<50,000/ mm3) was the most common "severe" manifestation (888/45,775 with pooled prevalence of 8.6%). The case fatality was 0.3% (353/46,411). In conclusion, *P. vivax* can cause severe and even fatal disease. More detailed epidemiological studies are needed which dissociate causation from association to refine the definition and estimate the prevalence of severe vivax malaria.

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COMPARISON OF METHEMOGLOBIN LEVEL BETWEEN CHILDREN AND ADULT AFTER TREATMENT WITH PRIMAQUINE

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Primaquine prevents relapse and sterilizes infectious sexual plasmodia, but confusion surrounds its use. It is able to convert hemoglobin to methemoglobin, producing cyanosis. Limited data support the influence of primaquine on level of methemoglobin in children. A prospective study part of a clinical trial was done in an endemic area of malaria, North Sumatera, Indonesia. Patients diagnosed with *Plasmodium vivax* were given ACTs and low dose primaquine for 14 days. Methemoglobin level was measured on day 0, 7 and 14. A descriptive analysis and un-paired ttest were carried out. Among 3168 patients that were screened, 331 (10.45%) was enrolled. We found 65.86% children and 34.14% adult. Mean level of Methemoglobin in children on day 0 was 1.75% compared to 1.53% in adult, p=0.03. On day 14, Mean methemoglobin level in children was 5.46% and adult 4.76%, p=0.03. 12/218 (5.5%) of children had methemoglobin level > 10% compared to adult (5.3%) 6/113. The highest methemoglobin level (19.6%) was found in a child on day 14, fortunately without any symptoms. An increase level of methemoglobin found in malaria patients in North Sumatera, Indonesia after receiving treatment of primaquine. Children were more prone to methemoglobinemia compared to adult.

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HEALTH SYSTEM STRENGTHENING

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Kisumu is one of the top 15 counties with poor maternal and neonatal health indicators. Malaria in pregnancy (MIP) is one of the conditions associated with poor pregnancy outcomes including maternal anaemia, miscarriages, low birth weight and neonatal deaths. However, the uptake of the effective interventions like intermittent preventive treatment of malaria in pregnancy using sulfadoxine pyrimethamine (IPTp-SP) has remained belowthe national targets at 50% for 2 or more doses and 33% for 3 or more doses in areas providing IPTp.To enable the country move towards achieving the national targets,ministry of health has developed simplified guidelines on how to prevent and treat malaria in pregnancy and how to create demand for health services by communities. To build the capacity of Muhoroni subcounty to scale up the effective MIP interventions a) health care workers (HCWs) in public, faith-based organizations, community-based organizations and private sector providing antenatal care services (ANC) services were trained on provision of the interventions b) community health volunteers (CHVs)were trained on MIP messaging at community levelto sensitize pregnant women and create demand for health services. A total of 264 out of 312 (84.6 %) HCWs in 30 out of 32 (93.8 %) facilities providing ANC were trained. A total of 318 out 340 (93.5 %) targeted CHVs in all 34 community units were trained on sensitization of pregnant women on importance of adhering to scheduled ANC visits. To reduce the poor maternal and neonatal health indicators associated with effects of malaria in pregnancy, it is important to scale up the coverage rates of MIP interventions to the set national targets. The subcounty in an effort to increase coverage towards the national targets built the capacity of HCWs on prevention and treatment of malaria in pregnancy and CHVs on sensitization of pregnant women to attend scheduled ANC visits.

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REDUCTIONS IN MALARIA IN PREGNANCY AND ADVERSE BIRTH OUTCOMES FOLLOWING INDOOR RESIDUAL SPRAYING OF INSECTICIDE IN UGANDA

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Indoor residual spraying of insecticide (IRS) is a key intervention for reducing the burden of malaria in Africa. However, data on the impact of IRS on malaria in pregnancy and birth outcomes is limited. We conducted an observational study nested within a trial of intermittent preventive therapy during pregnancy in Tororo, Uganda. Women were enrolled at 12-20 weeks of gestation between June-Oct 2014, provided with insecticide treated bednets, and followed through delivery. From Dec 2014-Feb 2015, carbamate-containing IRS was implemented in Tororo district for the first time. Exact spray dates were collected for each household. The exposure of interest was the proportion of time during a woman's pregnancy under protection of IRS. Of 289 women followed, 134 had no IRS protection during pregnancy, 90 had >0-20% IRS protection, and 65 had >20-43% protection. During pregnancy, malaria incidence (0.49 vs 0.10 episodes ppy, P=0.02) and parasite prevalence (20.0% vs 8.9%, P<0.001) were both significantly lower after IRS. At the time of delivery, the prevalence of placental parasitemia was significantly higher in women with no IRS protection (16.8%) compared to women with 0-20% (1.1%, P=0.001) or >20-43% IRS protection (1.6%, P=0.006). Compared to women with no IRS protection, those with >20-43% IRS protection had a lower risk of LBW (20.9% vs 3.1%, P=0.002), preterm birth (17.2% vs 1.5%, P=0.006), and fetal/neonatal deaths (7.5% vs 0%, P=0.03). In cocnlusion, in this setting, IRS was temporally associated with lower malaria parasite prevalence during pregnancy and at delivery, and improved birth outcomes.

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HIGH-THROUGHPUT METABOLOMICS TO IDENTIFY BIOMARKERS OF RELAPSES IN *PLASMODIUM VIVAX*-INFECTED PATIENTS

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Plasmodium vivax is the most widely distributed cause of human malaria worldwide, and presently more than 40% of the global population is at risk of infection with this parasite. *P. vivax* develops latent stages in the liver, where the hypnozoites can generate new acute malaria episode months or years after the initial infection. Recently, systems biology and mathematical modeling studies have highlighted the role of relapses in *P. vivax* infection in geographical areas where the parasite is transmitted. Therefore, the new agenda of malaria eradication includes controlling relapses in *P. vivax* infected patients. Although this is an important challenge towards eradication, a differential diagnosis for relapse in patients is currently unavailable. Thus, the aim of this study was to identify human metabolites that are differentially expressed in relapse compared to the primary infection in the same individuals. To reach this goal, high throughput untargeted metabolomics was performed on serum samples from individuals with clinical cases of initial infection and relapses. More than 2,800 metabolites features were detected using this platform, and 86 metabolites showed significant differences between the two groups (primary x relapse). Significant metabolites included inosine and taurine, and the former increased in relapses and the latter decreased in relapses. These metabolites can serve as potential biomarkers of relapse in *P. vivax* infection, and confirmation of their identities will determine their full potential.

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NEW EFFORTS AIMED AT REPLACING ARTEMISININS FOR MALARIA TREATMENT: IDENTIFICATION OF NOVEL, DRUG-LIKE AND FAST-ACTING COMPOUNDS WITH TRANSMISSION BLOCKING POTENTIAL FROM WITH PHENOTYPIC SCREENING

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Malaria is a mosquito-borne protozoal infection caused by parasites of the genus Plasmodium. Despite efforts to eradicate malaria in the past century, the disease remains a major global health problem. Nowdays, according to the 2014 World Health Organization (WHO) global malaria report, 3.3 billion people are at risk of being infected with malaria. From the 198 million cases reported in 2013 the disease led to ca. 500,000 deaths.[1] Plasmodium has been able to adapt to the different treatments developed by humans along history. Part of it as a consequence of its complicated life-cycle which involves resistance to the current first line treatments Artemisins Combination Therapies (ACT) is also arising. The wide knowledge of the illness and the awareness of governments/health systems/funding agencies, makes the current time a unique opportunity to change the course of this disease and achieve eradication. GSK efforts are focused on identifying quality novel chemotypes suitable for oral administration and activity versus strains resistant to current antimalarials in the clinic. Ideally that new molecule should present activity versus the parasite forms involve in the transmission of the disease. [2,3] Therefore, we are seeking the next generation of antimalarials. In this communication we will discuss our latest advances in the field. References [1] World Health Organization. World malaria Report 2014; WHO Press: Geneva, Switzerland, 2014. http://www.who.int/malaria/ publications/world_malaria_report_2014/report/en/ (accessed August, 4, 2015). [2] F. J. Gamo, et. al., Nature 2010, 465, 305-312.; [3] J. Lelièvre et al, PLoS ONE 2012, 7(4), e35019 The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents. All animal studies were ethically reviewed and carried out in accordance with European Directive 86/609/EEC and the GSK Policy on the Care, Welfare and Treatment of Animals.

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TRENDS IN U.S. PRESIDENT'S MALARIA INITIATIVE-FUNDED INDOOR RESIDUAL SPRAY COVERAGE AND INSECTICIDE CHOICE IN SUB-SAHARAN AFRICA (2008-2015): URGENT NEED FOR AFFORDABLE, LONG-LASTING INSECTICIDES

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The changing pattern of US President's Malaria Initiative-funded IRS in sub-Saharan Africa between 2008-2015 was studied. IRS coverage in sub-Saharan Africa increased from <2% of the at-risk population in 2005, to 11% or 78 million people in 2010, mainly as a result of increased funding from PMI. The scaling up of IRS in sub-Saharan Africa has been successful in several epidemiological settings. However, the spread and intensification of pyrethroid resistance in malaria vectors led many control programmes to spray alternative insecticides. Between 2009-2013, pyrethroid spraying decreased from 87% (13/15) of PMI-funded countries conducting IRS to 44% (7/16), while bendiocarb use increased from 7% (1/15) to 56% (9/16). Long-lasting pirimiphos-methyl CS received WHOPES recommendation in 2013 and was scheduled to be sprayed in 85% (11/13) of PMI-funded countries conducting IRS in 2015. The gradual replacement of relatively inexpensive pyrethroids firstly with bendiocarb (carbamate) and subsequently with pirimiphos methyl CS (organophosphate) has contributed to downscaling of most PMI-funded IRS programmes. Overall, there was a 53% decrease in the number of structures sprayed between years of peak coverage and 2015, down from 9.04 million to 4.26 million structures. Sizeable reductions in the number of structures sprayed were reported in Madagascar (56%, 576,320

to 254,986), Senegal (64%, 306,916 to 111,201), Tanzania (68%, 1,224,095 to 389,714) and Zambia (63%, 1,300,000 to 482,077), while in Angola, Liberia and Malawi PMI-funded spraying was suspended. The most commonly cited reason was increased cost of pesticides, as vector resistance necessitated switching from pyrethroids to organophosphates. There are worrying preliminary reports of malaria resurgence following IRS withdrawal in parts of Benin, Tanzania and Uganda. At present, there are several countries reliant on organophosphates and carbamates for IRS and increasing resistance is a serious threat that could result in IRS no longer being viable. A portfolio of new cost-effective insecticides with different modes of action is urgently needed.

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SENTINEL-SITE SURVEILLANCE FOR MALARIA MORTALITY AND ITS POTENTIAL FOR EBOLA VIRUS DISEASE OUTBREAK DETECTION IN GUÉCKÉDOU, GUINEA

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Community-based surveillance systems are used to document program impact on mortality from diseases such as malaria. They could also act as surveillance systems for outbreak detection. We conducted a retrospective analysis of data from a prospective malaria mortality surveillance system in Guéckédou, Guinea through which data was reported monthly from July 2011-July 2014. In 46 sentinel sites, 40 with a malaria control program (program) and 6 without (comparison), cause of death as reported by the deceased's family was recorded by key informants. Deaths were classified as due to malaria or another cause. Suspect Ebola virus disease (EVD) deaths were those reported as due to symptoms compatible with the EVD case definition. Deaths were aggregated by area and analyzed by 6 month surveillance period (1-6) corresponding to the dry (January-June) and rainy (July-December)seasons. Logistic regression models were used to investigate temporal trends in malaria-attributable mortality and suspect EVD mortality. From July 2011-June 2014 approximately 45,000 individuals were monitored by the surveillance system, 1,242 deaths were reported. The majority (55.2%, n=686) were reported as due to malaria. Proportional mortality attributable to malaria decreased from 66.8% to 40.3% ($p<0.001$) in the program area and from 65.8% to 59.2% ($p=0.782$) in the comparison area. 75.2% (n=934) of all deaths occurred at home, 17.8% (n=221) occurred in a health facility. In total, 68 deaths [range 6-17, by period] were classified as EVD suspect and increased over time ($p=0.021$). Seventeen suspect EVD deaths were reported during period 6, January-June 2014, when EVD was detected; these included the first two (11.7%, 2/17) laboratory confirmed EVD deaths in the region. Community surveillance can be used to document program impact on mortality and complement health facility surveillance particularly in areas where the majority of deaths occur in the community. Such systems could also be used for outbreak detection. Had community surveillance for outbreak detection been in place, the EVD outbreak in Guéckédou might have been identified earlier.

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BUILDING CAPACITY TO ACCELERATE IPTP UPTAKE THROUGH THE ADOPTION OF 2012 WHO IPTP GUIDANCE IN MALAWI

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Malawi adopted the World Health Organization's updated guidance on intermittent preventive treatment in pregnancy (IPTp) in 2013. Support from the US President's Malaria Initiative through USAID funded health projects, enabled collaboration between the National Malaria Control Program (NMCP) and the Reproductive Health Directorate (RHD) of the Ministry of Health, to build capacity from national to district to frontline health facility levels to implement the updated IPTp policy. These partners updated IPTp policy in the National Malaria Treatment Guidelines, and developed appropriate training manuals. All 5708 health workers from the 304 facilities in the 15 project districts were trained on the IPTp policy and guidelines. Post-training test scores of health staff increased over pre-test by an average of 40 percentage points. The community action cycle approach engages community volunteers and local community based organizations to identify and solve local problems and was used to encourage pregnant women to attend antenatal care (ANC) and receive IPTp and long lasting insecticide-treated nets. Health information system data from the 15 Districts were used to compare ANC and IPTp coverage for 2012 and 2015 fiscal years (Oct.-Sept.). ANC registration in the project area rose from 113,683 to 394,116. IPTp1 as a proportion of ANC registration rose from 52% to 87%, and IPTp2 increased from 17% to 62%. While IPTp3 doses were recorded in the ANC registers, reporting forms in 2015 still did not include space to enter this IPTp3. Observations at clinics showed IPTp3 and 4 were provided. Malawi's experience shows that collaboration between NMCP and RHD as well as between clinics and communities not only disseminated knowledge of the new policy, but resulted in increased uptake of services and protection of pregnant women from malaria.

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ACCURATE MALARIA MICROSCOPY: IS IT THE READER OR QUALITY OF PREPARED SMEARS? A QUANTITATIVE COMPARISON OF FALSE POSITIVES/NEGATIVES VS SMEAR QUALITY IN TANZANIAN MILITARY HEALTH FACILITIES

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Quality malaria microscopy remains the gold standard for malaria diagnosis. For the past 5 year WRAIR/TPDF has implemented innovative strategies to increase access to quality malaria case management through monitoring and improving quality of malaria microscopy. The accuracy and reliability of results are critically dependent on the quality of the blood film preparation, the staining procedure and the microscopist's expertise in reading the smears. Studies have demonstrated poor malaria microscopy performance in Africa, including Tanzania. Walter Reed and Tanzania Peoples Defence Forces (TPDF) have established a Quality Monitoring and Improvement Program. The key objective is to perform crosschecking of malaria slides prepared and examined in current and future research sites. All testing sites are provided with high quality equipment, reagents and supplies to perform malaria microscopy. All blood films prepared for routine testing are stored and a monthly QC sample is crosschecked by the Walter Reed Malaria Program. The QC samples are selected in situ from the laboratory register on a quarterly basis. 10 negative and all positive slides for each month are selected using a random representative sampling protocol. The quality of blood films are quantitatively assessed macroscopically for blood film preparation quality and microscopically for staining quality and reading accuracy. The quantitative assessment of preparation, staining and reading shows correlation between the quality of the smear and accuracy of the results reported. In this study we will

present the data on the trend of false positive rates as compared to increase or decrease on the total quality index of preparation and staining of blood smears over the past 12 months.

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A CROSS-SECTIONAL SURVEY OF KNOWLEDGE AND PERCEPTIONS RELATED TO PREVENTION AND TREATMENT OF MALARIA IN PREGNANCY AMONG HEALTH CARE PROVIDERS AT HEALTH FACILITIES IN TANZANIA

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A cross-sectional survey was conducted among 131 health-care providers in rural Tanzania to examine the knowledge and availability of malaria preventive and curative treatment for pregnant women recommended by the World Health Organization and contained in Tanzanian national guidelines. Perceptions of harm attributable to malaria infection and, separately, to malaria treatment were recorded, as well as malaria-specific training received in the last three years. Thirty-nine percent of providers correctly stated that malaria during the first trimester should be prevented by sleeping under insecticide treated nets only. In contrast, 80% of providers correctly reported that malaria is to be prevented using insecticide treated nets and intermittent preventive treatment of malaria with sulfadoxine-pyrimethamine during the second and third trimesters. Knowledge of appropriate first-trimester case management was correctly reported by 50% of providers, increasing to 60% when providers were asked to describe curative care for pregnant women in their second and third trimesters. Approximately 30% of the providers stated having received malaria training in the last three years. Untreated malaria infection was viewed as extremely harmful by over 93% of providers to pregnant women and their unborn babies in any trimester. Malaria treatment was considered harmful to pregnant women in the first trimester and unborn babies in the second and third trimesters by 44% and 45% of providers, respectively. Only 48%, 44% and 69% of providers reported having sulfadoxine-pyrimethamine, quinine, and artemisinin-combination treatment available at the health facility, respectively, for preventive and curative care. These results suggest that the scale up of interventions to reduce the burden of malaria in pregnancy will, in part, require substantial improvements in supply chain management as well as pre-service training and in-service retraining of health-care providers in appropriate preventive and curative care with an emphasis on the safety of treatments by trimester.

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DEVELOPMENT OF *PLASMODIUM FALCIPARUM* EXFLAGELLATION ASSAY

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The standard membrane-feeding assay (SMFA) is considered a gold standard functional assay for transmission-blocking vaccine (TBV) development against *Plasmodium falciparum* malaria. However, it has been shown that SMFA reproducibility is poor when % inhibition of oocysts is low. In addition, the assay needs an insectary which can provide *Anopheles* mosquitoes and fulfill all regulatory requirements to maintain infectious mosquitoes. Therefore, we evaluated an exflagellation assay (EXA) for assessment of functional activity of transmission-blocking antibodies. Since EXA does not involve parasite development process in mosquitoes, it is possible that EXA could show better precision than SMFA. Two monoclonal antibodies (mAbs), 1B3 (anti-Pfs230) and IIC5B10 (anti-Pfs48/45), both of which have shown strong functional activities in SMFA, were utilized for the assay development. We first optimized the protocol of EXA, e.g., ratio of test antibody and gametocyte culture, incubation time. In the optimized EXA, clear enhancement of inhibition was observed in the presence of complement for both mAbs, while IIC5B10 mAb does not require complement to work in SMFA. We confirmed the specificity of inhibition using 4B7 mAb, which recognizes Pfs25, one of the post-fertilization TBV candidates. Both 1B3 and IIC5B10 mAbs showed dose-dependent inhibition in the EXA, and the inter-assay precision of IIC5B10 mAb was much better in EXA than in SMFA. The results indicate that EXA could be one option to evaluate functional activity of transmission-blocking antibodies which target pre-fertilization antigens.

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SAFETY AND IMMUNOGENICITY OF WITH NOVEL MALARIA VACCINE CANDIDATE, R21 ADJUVANTED WITH MATRIX M1

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Falciparum malaria remains one of the leading infectious causes of morbidity and mortality worldwide. Development of a durable efficacious vaccine is a priority, and there remains an urgent need to improve efficacy to achieve the World Health Organisation goal of a deployable vaccine with at least 75% durable efficacy against clinical malaria by 2030. R21 has been developed at the Jenner Institute, University of Oxford. This is an improved RTS,S construct, an antigen derived from the pre-erythrocytic circumsporozoite protein, which is an abundant coat protein involved in sporozoite development and hepatocyte invasion. R21 comprises recombinant particles expressing the central repeat and the C-terminus of the circumsporozoite protein (CSP) fused to HBsAg, but without the excess of unfused HBsAg protein found in RTS,S. It was GMP manufactured in Pichia pastoris at the Clinical Biomanufacturing Facility at Oxford University. We undertook a Phase I, open-label clinical trial to assess the safety and immunogenicity of R21 administered alone (Group 2; n=4) and with the novel saponin-based adjuvant, Matrix M1 (Group 1 and 3; n=10 per group). Safety was assessed by active and passive collection of local and systemic adverse events. The immunoassay of most interest was the antibody response to NANP because this correlates with vaccine efficacy after RTS,S/AS01 administration, and induction of antibody levels comparable to or greater than RTS,S/AS01 would suggest likely vaccine efficacy. Preliminary analysis shows that the vaccine is safe and well tolerated with the majority of adverse events being mild in nature and short-lived. Initial assessment of antibody responses indicate antibody levels elicited at least as high as reported for RTS,S/AS01 in previous studies. This trial was the first administration of R21 in humans and safety and immunogenicity profiles observed support further testing in Phase IIa studies using a controlled human malaria infection model.

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PHASE I VACCINE TRIAL FOR EBA-175 RII INDUCES HIGH LEVELS OF BINDING INHIBITORY ANTIBODIES THAT TARGET KEY FUNCTIONAL EPITOPES

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Naturally acquired antibody responses against blood-stage parasites are associated with protective immunity. Erythrocyte Binding Antigen-175 (EBA-175) is a leading vaccine candidate of *Plasmodium falciparum* that plays a key role in merozoite invasion by binding to glycophorin A on the erythrocyte surface. We have previously shown that antibodies to EBA-175 are associated with protection and can inhibit EBA-175 binding to glycophorin A. This binding is mediated through Duffy-binding like (DBL) domains in Region II of the protein. EBA175 Region II was recently assessed in a dose escalation Phase I trial adjuvanted with Alum and individuals (n=71) were shown to develop high titres of IgG but relatively low growth inhibitory responses. Our study aimed to further assess the properties and functional activity of these vaccine-induced antibodies and relate the magnitude of these responses with antibodies acquired following natural infection in a cohort of children from Papua New Guinea (n=206). There were no statistically significant differences in total IgG levels between the vaccine-induced and naturally acquired responses (p=0.883). However, vaccine-induced responses were significantly lower compared to naturally-induced responses for IgG1 (p=0.003) and IgG3 (p<0.001); both key subclasses associated with protection from symptomatic disease in naturally immune populations. Vaccine induced antibodies were able to inhibit EBA-175 binding, especially for the 20µg per dose or greater. Furthermore, the vaccine was able to elicit antibodies that targeted epitopes shared with the functional monoclonals, R217 and R218. Importantly, there was a high correlation between binding inhibitory activity and antibodies to the R217 epitope (0.8860; p<0.0001). This study demonstrates that a recombinant EBA-175 vaccine can induce high level binding inhibitory antibodies that target key epitopes on EBA-175 Region II however the differences in subclass responses requires further optimisation. The exploration of adjuvants is required to ensure that the vaccine-induced immunity is optimised further and exceeds natural immunity.

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PROTEOMIC ANTIBODY PROFILING OF U.S. AND AFRICAN VOLUNTEERS IN MULTIPLE CLINICAL TRIALS OF PFSPZ VACCINE

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A vaccine against malaria would benefit the millions affected worldwide and accelerate efforts to eliminate malaria. Immunization with PfSPZ Vaccine, a metabolically active, non-replicating malaria sporozoite vaccine, induced high-level protective efficacy against *Plasmodium falciparum* (Pf) malaria infection in multiple clinical trials. An antibody assay that predicted protection would facilitate vaccine development and deployment. To identify the target proteins for such an assay, we developed whole Pf proteome microarrays representing 4,805 unique genes, or approximately 91% of the Pf proteome, to screen sera from volunteers in clinical trials of PfSPZ Vaccine who underwent controlled human malaria infection (CHMI) or followed for risk of malaria. Sera have been assessed for proteomic antibody profiles in three clinical trials of U.S. volunteers and clinical trials of African volunteers from Mali and Tanzania. We have detected over 1,500 responses to Pf proteins in PfSPZ Vaccine recipients. Over 50 Pf proteins have been identified with an immunogenic profile and association with sterile protection after CHMI. A combination of 6 proteins (PfCSP, PfMSP5, PfGSK3, PfLRR9, PfDOC2 and PF3D7_1030200) that predicted protection was identified in one clinical trial with 32 U.S. volunteers, which had a cross-validated AUC of 0.89 and sensitivity and specificity of 92% and 89%, respectively. This signature of protection has been confirmed in an independent clinical trial. These 6 Pf proteins were selected for further development as companion assays to PfSPZ Vaccine for travelers and potential components of subunit malaria vaccines. Unique antibody profiles were observed between trials of PfSPZ Vaccine in U.S. and African volunteers. A signature of protection is being investigated for African PfSPZ Vaccine recipients for development of a companion assay to predict when subjects will be protected during malaria elimination.

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A HIGHLY INFECTIOUS *PLASMODIUM YOELII* PARASITE, BEARING PLASMODIUM CIRCUMSPOROZOITE PROTEIN

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Plasmodium circumsporozoite protein (CSP) is a major surface antigen present in the sporozoite (Spz) stage of a malaria parasite. RTS,S vaccine, the most clinically advanced malaria vaccine, consists of a large portion of *Plasmodium falciparum* CSP (PfCSP). A highly infectious, recombinant rodent malaria, *Plasmodium yoelii* parasite bearing a full-length PfCSP (PfCSP/Py) was generated by double cross-over homologous recombination. This PfCSP/Py parasite produced up to 30,000 Spz in mosquito salivary glands, which is equal or even higher than the number of Spz produced by wild-type *P. yoelii* parasites. Five bites of PfCSP/Py-infected mosquitoes could induce blood infection in BALB/c mice. Our new transgenic parasite that expresses a full-length PfCSP may become a useful tool for researchers to investigate immunity against PfCSP in a mouse model.

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VECTORED PFCSP VACCINES BASED ON BACULOVIRUS DUAL EXPRESSION SYSTEM AND ADHU5 INDUCE STRONG PROTECTIVE EFFICACY AGAINST TRANSGENIC *PLASMODIUM BERGHEI*

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The baculovirus-vectored vaccine based on the "baculovirus dual expression system (BDES)" has been exploited as a novel vaccine platform for malaria. The gene encoding the human decay-accelerating factor was incorporated into the BDES malaria vaccine expressing the *Plasmodium falciparum* circumsporozoite protein (PfCSP). The newly developed BDES vaccine "BDES-sPfCSP2-Spider" resulted in complement resistance both *in vitro* and *in vivo*. To improve the vaccine efficacy, baculovirus expressing mouse interleukin-12 (mIL-12) and the adenoviral vaccine expressing PfCSP "AdHu5-sPfCSP2" were generated. Large-scale immunization studies were conducted in mice, and the protective efficacy was examined by using biting of mosquitoes infected with transgenic *P. berghei* sporozoites expressing PfCSP. After the priming immunization with AdHu5-sPfCSP2, booster immunization with BDES-sPfCSP2-Spider together with the mIL-12 vector conferred strong protective efficacy as compared to the controls (29 mice out of 44 were protected; 65%), following the high level of anti-PfCSP IgG titer. Thus, we propose that the prime-boost regimen using adenovirus and BDES offer great potential as a new malaria vaccine platform.

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MALARIA TRANSMISSION-BLOCKING VACCINE ANTIGEN DISCOVERY USING NATURALLY ACQUIRED FUNCTIONAL ANTIBODY

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Transmission-blocking vaccines (TBV) target the *Plasmodium falciparum* parasite's sexual stages to interrupt its life cycle and would be useful for elimination efforts. We previously identified human sera from Malian adults whose purified IgG conveyed high transmission-blocking activity (TBA) by standard membrane feeding assay (SMFA) against laboratory cultured gametocytes fed to A. stephensi mosquitoes. Here, we describe the results from an iterative subtractive screening of a gametocyte stage cDNA phage display library using naturally acquired IgG with versus without TBA. A set of novel TBV candidate antigens was identified including 3 proteins with hits in four independent differential screens. The top 9 candidates are being evaluated in an animal model using DNA immunization via gold particle bombardment, and the top 3 mentioned above using protein immunization as well. Briefly, synthetic genes were cloned into pCI-SF mammalian expression vector and pET-24b(+) for *E. coli* protein expression, respectively. Mammalian cell transient transfection was used to assess expression from pCI-SF clone's prior to animal immunizations. Independently, cobalt affinity column was used for protein purification from pET-24b(+) bacterial expression. Protein and DNA immunogens are being used to immunize small animals, and functional immune responses evaluated by SMFA will be reported.

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ASSOCIATION OF SPECIFIC VAR2CSA HAPLOTYPES WITH WORSENED BIRTH OUTCOMES IN WOMEN WITH *PLASMODIUM FALCIPARUM* PLACENTAL MALARIA IN MALAWI AND BENIN

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Pregnancy associated malaria (PAM) causes adverse pregnancy and birth outcomes including low birth weight (LBW) and small for gestational age (SGA). Placental accumulation of *Plasmodium falciparum* is mediated by VAR2CSA. This protein's ID1-DBL2x region is considered a minimal binding epitope and is a promising vaccine candidate against PAM. We hypothesized that variation in the ID1-DBL2x region would be associated with differential prevalence of poor birth outcomes. Using two clinical cohorts of women with placental malaria at delivery, we deep-sequenced the 1.6kb ID1-DBL2x region in 101 placental samples in Malawi and Benin to characterize genetic diversity and identify pathogenic clades. In Malawi, we identified two genetic clades which resembled the sequences of the current vaccine candidate antigens, 3D7 & FCR3. In Benin, along with 3D7-like and FCR3-like clades, three other unique clades were detected.

We estimated the association of specific clades with birth weight, LBW, and SGA, controlling for confounders using inverse probability weights. In our study population, the mean (SD) infant birth weight in Malawi was 2677g (540g) and 2840g (380g) in Benin. Prevalence of LBW was 19.6% (n=11) in Malawi and 13.3% (n=6) in Benin; prevalence of SGA was 16.1% (n=9) in Malawi and 24.4% (n=11) in Benin. In phylogenetic analyses, the variants present in the placentae of women delivering LBW or SGA infants clustered more readily in the 3D7-like clade in Malawi but were more evenly distributed in Benin. Compared to women infected with FCR3-like only variants, women infected with 3D7-like only variants delivered infants with lower birth weight (-267.99g; 95% CI: -466.43g - -69.55g) and higher odds of LBW (OR: 8.19; 95% CI: 1.65 - 40.57) and SGA (OR: 3.65; 95% CI: 1.00 - 13.38). These associations were attenuated in Benin, but were overall supported by country-level analyses. The results from our study provide evidence that 3D7-like genetic variants of VAR2CSA in parasites infecting the placenta are associated with worse birth outcomes including LBW and SGA. This supports the development of polyvalent vaccines that target multiple clades of VAR2CSA to combat PAM.

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EFFICACY OF PFSPZ VACCINE AGAINST HETEROLOGOUS MALARIA CHALLENGE IN MALARIA-NAÏVE ADULTS

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Sterile protection lasting 14 months against *Plasmodium falciparum* (Pf) malaria has been achieved in humans after intravenous (IV) injection of a non-replicating, cryopreserved Pf sporozoite (SPZ) vaccine (PfSPZ Vaccine). Further development is focused on identification of a dose-sparing immunization regimen that induces durable protection against heterologous Pf strains. We conducted an open-label trial of PfSPZ Vaccine, composed of attenuated, aseptic, purified cryopreserved PfSPZ, at a dose of 9.0 x105 PfSPZ administered IV 3 times at 8-week intervals to 15 healthy, malaria-naïve adults. Vaccinated and non-vaccinated control volunteers underwent controlled human malaria infection (CHMI) by exposure to mosquitoes carrying infectious PfSPZ of homologous 3D7 and heterologous 7G8 Pf strains at 19 and 33 weeks, respectively, after final immunization. Antibody and cellular immune responses were assessed. PfSPZ Vaccine was well tolerated. After CHMI with homologous PfSPZ at 19 weeks, 9/14 volunteers (64%) remained without parasitemia compared to 0/6 controls (P=0.012, Fisher's exact test, one-sided). Six non-parasitemic volunteers underwent repeat CHMI with heterologous PfSPZ at 33 weeks, and 5/6 vaccinees remained without parasitemia compared to 0/6 controls (P=0.0076). Pf-specific antibody, CD8, CD4, and γδ T cell responses were detected in all vaccinees. A 3-dose regimen of PfSPZ Vaccine conferred sterile protection for at least 33 weeks against CHMI with heterologous Pf and induced broad-based PfSPZ-specific immune responses. Ongoing studies using higher doses are evaluating protective efficacy in travelers, military personnel, and infants and adults living in endemic areas.

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IMMUNIZATION BY MOSQUITO BITE WITH RADIATION ATTENUATED SPOROZOITES (IMRAS): A PHASE 1 CLINICAL TRIAL

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Human clinical trials have demonstrated that immunization with radiation-attenuated *Plasmodium falciparum* sporozoites (PfRAS) by mosquito bite is an excellent model for malaria vaccine development conferring the highest levels of sterile protection. In the study Immunization by mosquito bite with radiation-attenuated *P. falciparum* sporozoites, or IMRAS, we have applied a systems biology approach to this model to improve current understanding of immune mechanisms of protection by comparing sterilely protected with non-protected study subjects. The clinical study was designed such that approximately 50% of immunized human subjects would be protected against homologous controlled human malaria infection (CHMI) to facilitate the analysis of biomarkers and correlates of protection. Earlier studies suggested that immunization with a total of 960 bites from mosquitoes infected with PfRAS would yield 50% protective efficacy. The study was conducted with two sequential cohorts, each consisting of twelve true-immunized and four mock-immunized human subjects. Subjects in both cohorts underwent five immunization sessions every four weeks receiving approximately 200 infectious bites per immunization session. Immunization procedures were well-tolerated, and there were no vaccine-related serious adverse events. All twelve infectivity controls (six per cohort) became parasitemic and none of the mock-immunized subjects were protected. Surprisingly, despite the similar number of total infectious bites in each cohort, the percentage of subjects protected in the two cohorts was quite different. Six of the 11 (55%) true-immunized subjects in the first cohort were sterilely protected against parasitemia after primary challenge at 23-25 days post-last immunization. In the second cohort, 9 of 10 (90%) true-immunized subjects were protected after primary challenge. We will present a detailed analysis of all factors which may have impacted the discordant levels of protective efficacy in the two cohorts; such data may provide key information for the development of a highly protective malaria vaccine.

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TOLERABILITY, SAFETY AND EFFICACY (ADULTS) OF ESCALATING DOSES OF PFSPZ VACCINE ADMINISTERED BY DIRECT VENOUS INOCULATION TO TANZANIAN INFANTS, CHILDREN, ADOLESCENTS AND ADULTS

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PfSPZ Vaccine administered by direct venous inoculation (DVI) has been shown to be extremely well tolerated and safe and induce durable, sterile protection against homologous and heterologous *Plasmodium falciparum* (Pf) parasites in controlled human malaria infection (CHMI)(U.S. and Tanzania) and field (Mali) studies in doses up to $2.7x10^5$ Pf sporozoites (SPZ). However, while good, the protective efficacy has not yet been shown to achieve our target of at least 80% sterile protection for at least 6 months, and PfSPZ Vaccine has not yet been studied in adolescents, children, or infants. All current data indicate that increasing the numbers of PfSPZ per dose will increase protective efficacy. To address both deficiencies, we are conducting a double blind, normal saline placebo-controlled trial at the Bagamoyo Clinical Trials Unit of the Ifakara Health Institute in Tanzania. The trial is aimed at determining if 3 doses of up to $9.0x10^5$ PfSPZ (young children and infants) and $1.8x10^6$ PfSPZ (adults, adolescents, older children) are well tolerated, safe and immunogenic and protect adults against CHMI. 93 female and male subjects were enrolled in an approximately 2:1 ratio (vaccinee to control) in the five age groups including 18 volunteers in each of the adult, 11-17, 6-10, and 1-5 year age groups, and 21 in the 6-11 months group, plus an additional 6 adults to serve as infectivity controls for CHMI. Two of the three planned doses have been administered to adults, adolescents, and older children and 50% of the younger children and infants. The study is still blinded. However, there have been no serious adverse events, nor any indication of differences in adverse events among the age groups, or of a safety signal. Adults will undergo CHMI by DVI of PfSPZ Challenge (aseptic, purified, infectious PfSPZ) in May and June 2016, and the study will be completed in September 2016. The complete un-blinded results of tolerability, safety, immunogenicity, and protective efficacy will be presented, as will plans for the next Tanzanian study designed to support licensure for the mass vaccine administration indication.

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CLINICAL MANIFESTATIONS OF *PLASMODIUM FALCIPARUM* INFECTION IN TANZANIAN ADULTS AFTER CONTROLLED HUMAN MALARIA INFECTION BY INJECTION OF PFSPZ CHALLENGE

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The clinical manifestations of Plasmodium falciparum (Pf) malaria in volunteers in the United States and Europe after controlled human malaria infection (CHMI) by exposure to mosquitoes carrying Pf sporozoites (SPZ) and by injection of aseptic, purified, cryopreserved, infectious Pf sporozoites (SPZ), a product called Sanaria® PfSPZ Challenge, have been well described. We conducted the first CHMI study in Africans using intradermal injection of PfSPZ Challenge; 21 subjects developed malaria. Since then we have conducted three different CHMIs by direct venous inoculation (DVI) of PfSPZ Challenge; 56 subjects developed malaria. In May and June 2016 we will conduct two more CHMIs by DVI of PfSPZ Challenge in 26 subjects. In general with the same dose of PfSPZ Challenge the pre-patent periods are longer and the clinical manifestations less in previously exposed Tanzanians as compared to subjects in the U.S. and Europe with no previous exposure to malaria. We will present a detailed analysis of the parasitological findings and the clinical manifestations in the Tanzanian subjects, and contrast them with those reported for non-immune subjects. These data will provide a foundation for groups in Africa to design, justify, and execute CHMIs to assess the effects of antimalarial drugs and vaccines, naturally acquired immunity, and genetic background on malaria.

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CHEMOPROPHYLAXIS VACCINATION (CVAC) WITH SANARIA® PFSPZ CHALLENGE AND PYRIMETHAMINE: PHASE 1 TRIAL TO DETERMINE SAFETY AND PROTECTIVE EFFICACY AFTER EXPOSURE TO ONLY *PLASMODIUM FALCIPARUM* LIVER STAGES

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Direct venous inoculation (DVI) of aseptic, purified, infectious *Plasmodium falciparum* (Pf) sporozoites (SPZ) (Sanaria® PfSPZ Challenge) under chloroquine (CQ) prophylaxis (Sanaria® PfSPZ-CVac approach) protected 100% (9/9) of malaria naive vaccinees against homologous controlled human malaria infection (CHMI), 9 weeks after the third and last dose. CVac-CQ exposes vaccinees to both liver and blood stage antigens, thus the contribution of each parasite stage to immunity is unclear. We are investigating the safety and protective efficacy of CVac using CQ and pyrimethamine chemoprophylaxis (liver stage only exposure) versus CVac-CQ. Upon determination in a pilot study with 2 subjects that pyrimethamine dosing at days 2 and 3 post PfSPZ Challenge inoculation was sufficient, the main study enrolled 12 subjects to receive CVac using CQ and pyrimethamine and 6 subjects to receive CVac using CQ only. Subjects completed 3 rounds of PfSPZ-CVac at 4 weeks intervals. Thus far, PfSPZ-CVac has been well tolerated with the majority of adverse events (AEs) being transient Grade 1 AEs, with one serious AE reported. No subjects developed parasitemia, detected by thick blood smear, or clinical malaria. Subpatent parasitemia detected by polymerase chain reaction (PCR), was seen on days 7 and 8 post PfSPZ Challenge inoculation in during CVac-CQ, but not in any subject receiving CVac with CQ and pyrimethamine. All eligible vaccinated subjects and 5 subjects in an infectivity control group will undergo CHMI with 3,200 PfSPZ Challenge in June 2016. Unblinded tolerability, safety, immunogenicity, and protective efficacy results will be presented. This is the first trial to evaluate whether exposure limited to live replicating liver stage parasites in humans results in the development of immunity against homologous CHMI.

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DEVELOPMENT OF A PFSPZ VACCINE REGIMEN TO PROTECT MILITARY PERSONNEL AGAINST *PLASMODIUM FALCIPARUM* INFECTION

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Plasmodium falciparum (Pf) sporozoites (SPZ) are the only immunogens ever shown to induce >90%, sterile protective immunity against malaria.

Sanaria® PfSPZ Vaccine, an aseptic, purified, cryopreserved radiation attenuated Pf sporozoite (SPZ) malaria vaccine administered by direct venous inoculation has been shown to be safe, well-tolerated, easy to administer, immunogenic and highly protective. In order to be utilized as a vaccine to protect deployed military personnel, the vaccine must have an efficacy of >80% against infection with heterologous strains of Pf for at least 6 months. A series of clinical trials conducted by the Naval Medical Research Center, Walter Reed Army Institute of Research and other members of the International PfSPZ Consortium have demonstrated a distinct dose response for protection induced by PfSPZ Vaccine. Gradually increasing the total amount of vaccine (administered in as few as 3 immunizations) has generated increasing levels of protection and anti-PfCSP antibody titers. After reaching >90% protective efficacy against short-term homologous controlled human malaria infection (CHMI) at 3 weeks post final dose, we set the next benchmarks to include protection against 1) long-term homologous CHMI (6 months post final dose), 2) short-term heterologous CHMI, 3) long-term heterologous CHMI and finally, 4) protection against *P. vivax*. In our most recently completed clinical trial, we had unprecedented success in reaching the first and second of these benchmarks. We expect to meet the third benchmark (protection against long-term heterologous CHMI) with one of the regimens being assessed in our newly initiated clinical trial. These include a 3 dose regimen with up to 4 times as many PfSPZ per dose (1.8x10^6) as in our previous study and a regimen with the highest dose given in humans thus far (2.7x10^6), followed by two doses of 9x10^5. We will present a detailed analysis of the dose response to PfSPZ Vaccine, preliminary results from our ongoing trial, plans to assess protection against *P. vivax* CHMI and plans for Phase 3 testing to support submission for FDA licensure.

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SAFETY, TOLERABILITY AND EFFICACY OF DOSE ESCALATING DIRECT VENOUS INOCULATION WITH RADIATION ATTENUATED *PLASMODIUM FALCIPARUM* NF54 SPOROZOITES (PFSPZ VACCINE) IN HEALTHY MALIAN ADULTS

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A double blind, randomized Phase 1/2 clinical trial is being conducted in Mali, West Africa to assess the safety, immunogenicity and protective efficacy of increased doses of Sanaria® PfSPZ Vaccine administered via direct venous inoculation (DVI) against natural malaria exposure and controlled human malaria infection (CHMI) in healthy 18-50 year old adults. The initial dose escalation pilot study (part A) started in January 2016. Five volunteers received 1 dose of PfSPZ Vaccine ($4.5x10^5$ PfSPZ) and five volunteers received 1 dose of PfSPZ Vaccine ($9.0x10^5$ PfSPZ) in a staggered manner. Following no identified safety concerns, 30 additional volunteers have received three doses of PfSPZ Vaccine ($18.0x10^5$ PfSPZ) at eight week intervals in the dry season. These 30 subjects will also undergo further evaluation, including examination of protective efficacy against homologous CHMI via PfSPZ Challenge in May/June 2016. Fifteen additional subjects will be enrolled as infectivity controls during CHMI. The targeted dose ($18.0x10^5$ PfSPZ Vaccine), having been shown to be safe and tolerable, is now being administered to a larger main cohort in a double blind, randomized, placebo controlled trial (part B) to examine the protective efficacy of the vaccine against naturally occurring infection. During the malaria transmission season (August-December), volunteers will be examined and blood smears obtained every 2 weeks for 20 weeks in total, with the primary efficacy endpoint being observation of a positive blood smear following receipt of third vaccination. Safety, tolerability, immunogenicity, and protective efficacy from the pilot phase (part A) and safety and tolerability from the main phase (part B) will be presented.

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ANTIBODY PROFILING BY PROTEIN MICROARRAY OF HUMAN VOLUNTEERS PROTECTED BY IMMUNIZATION WITH RADIATION-ATTENUATED *PLASMODIUM VIVAX* SPOROZOITES

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A randomized, single-blinded clinical trial was conducted with Duffy positive (Fy+; Pv susceptible) individuals (n=21) assigned to either radiation attenuated sporozoites (RAS) or control groups; 14 received bites from irradiated (150 ± 10 cGy) Pv-infected *Anopheles* mosquitoes (RAS) and 7 from non-irradiated non-infected mosquitoes (control). Fy- (Pv refractory) volunteers (n=7) were immunized with non-irradiated Pv-infected mosquitoes. Eight weeks after the last immunization, 19 Fy+ and Fy- volunteers received a controlled human malaria infection (CHMI) with non-irradiated Pv–infected mosquitoes. Nineteen volunteers completed 7 immunizations (12 RAS, 2 Ctl and 5 Fy-) and received a CHMI. Five of 12 (42%) RAS volunteers were protected compared with 0/2 controls. None of the Fy- volunteers developed infection by the 7th immunization or after CHMI. To test whether protection might also be associated with IgG profiles, serum samples from day (0), after each immunization dose and 60 days post-CHMI were probed on a custom protein microarray displaying 500 *P. vivax* sero-reactive antigens. We observed that, while Fy- volunteers respond vigorously to non-irradiated vaccine, the RAS vaccine appeared to be only weakly antigenic in Fy+ volunteers (despite being protective in 5/12 RAS volunteers). Nevertheless, we observed significant changes in antibody profiles between protected and unprotected RAS volunteers post-CHMI, consistent with similar studies in *P. falciparum* vaccinees. We conclude that, protection from *P vivax* challenge can be achieved with relatively low doses of RAS vaccine.

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CHANGING THE PARADIGM OF VACCINE DEVELOPMENT: TURNING THE TARGET PRODUCT PROFILE (TPP) ON ITS HEAD

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Vaccines are lacking for many complex pathogens such as parasites and fungi, may be only partially protective, or may not be approved for key populations like children and pregnant women. There is an exigent need to enhance the efficiency of vaccine development, to generate new and better products, and to protect vulnerable populations. The International PfSPZ Consortium is developing a vaccine against a "refractory" pathogen - the malaria parasite - on an efficient and accelerated time line, with plans to submit a Biologics License Application in 2018 for a sporozoite-based vaccine providing > 80% sterile protection against malaria infection for > 6 months. The principles we have followed may be of value to the field. 1) We have avoided a detailed target product profile (TTP), even though this is often considered the first step in development. We believe TTPs force thinking "inside the box" and suppress innovation. Rather, we have ignored conventional restrictions such as adherence to traditional manufacturing methods, storage conditions, and routes of administration, contending that vaccines have just 3 required attributes (our universal TPP):

safety, tolerability and efficacy. 2) We selected a vaccine platform based on established proof-of-concept for high-level protection in humans. While a prototype vaccine may stray from other practical norms, high-level protection trumps everything else as the cornerstone of success. 3) The logistics of vaccine manufacturing, storage, delivery and administration have been addressed head-on as bioengineering and clinical challenges that need to be overcome. 4) Optimizing the immunization regimen has required classic vaccinology studies to adjust dose size, number and interval, and to select the best route of administration. 5) Speed of development has been enhanced by flexible trial design, receptivity to new approaches, intense transparent collaboration, immediate data communication, and frequent consultation with regulatory authorities, all driving toward licensure. These concepts will be discussed with the goal of promulgating a new, highly effective paradigm for vaccine development.

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DETERMINANTS OF INSECTICIDE TREATED NET USE AMONG UNDER-FIVE CHILDREN IN NIGERIA

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The use of Insecticide-treated nets (ITN) is the most cost effective malaria preventive measure. However, malaria remains a big killer of children in Nigeria accounting for about 30% of the under-fives (U5) deaths. Despite massive distribution campaigns nationwide, ITN use among (U5s) is low. This study assessed factors influencing ITN use among U5s across households in Nigeria.We analyzed the 2013 Nigerian Demographic and Health Survey dataset with specific focus on ITN use in children. A total of 17,664 U5s who slept in households with at least one ITN the night prior to the survey were studied. We conducted binary logistic regression analysis to identify factors influencing ITN use by U5s in (1) households that have at least one ITN and (2) households with at least one ITN for every two people (universal coverage). Data analysis was conducted using STATA (version 14.0) and results were considered significant at $p < 0.05$. In households with ITN ownership, only 28.5% of U5s slept under the ITN the night before the survey. Significantly, children less than three years old, those who reside in urban areas, those from other regions of the country except the North East had higher odds of using ITN the night before the survey. Also, children from households with less than 7 members and those from the second and third wealth quintiles had significantly higher odds of using ITN. On the second regression model among households with universal coverage, U5s who reside in urban areas, those from other regions of the country except the North East and those from the second and third wealth quintiles still had significantly higher odds of sleeping under ITN the night before the survey.Our findings have programmatic implications for malaria control among U5s in Nigeria. Alongside sustained ITN distribution to improve ITN coverage, health promotion/education efforts targeting parents/guardians on the relevance of ITN use in malaria prevention among U5s should be emphasized. Interventions to improve ITN use should be intensified among rural dwellers, larger households, and residents of North-Eastern Nigeria.

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TRANS STAGE DYNAMICS OF MIDGUT MICROBIOTA OF *ANOPHELES ALBIMANUS* FROM THE PACIFIC COAST OF COLOMBIA

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Colombia is the second country in Latin America in number of malaria cases reported, with an average in the last decades of over 100,000 cases per year, but underreporting is presumed. Some bacteria of the *Anopheles* mosquitoes gut microbiota seem to be involved in blocking development of the *Plasmodium* parasites and are being studied as alternative paratransgensis approach. Little is known about the autochthonous microbiota of the Latin-American anopheline mosquitoes; therefore, in this study, the midgut microbiota of fourth-instar larvae and adults of the main Colombian malaria vector *An. albimanus* was characterized. The midgut microbiota of specimens collected in the locality of the Colombian Pacific Coast was analyzed by both, culture-dependent and Mi-Seq Illumina high-throughput technology. Higher bacterial species richness was detected in immature stages while various bacterial species were detected in both stages, larval and adult females. Gram positive bacteria predominated in larvae and similar number of Gram positive and Gram negative bacteria were detected in adults. Current metagenomics analysis will complement the information on the bacterial community composition and their dynamics within the different stages of this important *Anopheles* vector species. This knowledge provides the basis for the design of new and effective vector interventions to control malaria transmission.

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FACTORS ASSOCIATED WITH OWNERSHIP OF INSECTICIDE TREATED NETS IN HOUSEHOLDS IN UGANDA

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Malaria is still a global public health challenge and in Uganda, it accounts for 40% of hospital outpatient visits, 20% of hospital admissions, and 14% of hospital deaths. Use of Insecticide treated nets (ITNs) is effective for malaria prevention and control in households. Universal coverage of ITNs (defined as one ITN for two people regardless of age or gender) is recommended for all households to achieve impact. Mass ITNs distribution campaigns remain a cornerstone in efforts to achieve universal coverage. As such, Uganda recently conducted a mass ITN distribution campaign, distributing over 33 million nets country wide. We examine factors associated with universal ownership of ITNs among households in Uganda to guide programmatic planning. We analyzed the Uganda Malaria Indicator Survey data collected in 2013/14. Factors associated with universal ownership of ITNs in households were examined using logistic regression. Independent variables assessed included area of residence, age and sex of household head, household socio-economic class, number of rooms in the household, and whether the household had odd or even number household size. Households in the lowest socio-economic class (OR = 0.641, p = 0.00) were less likely to have universal ownership of ITNs compared to those in the highest socio-economic class. Households with household size as an odd number (OR = 0.600, p = 0.00) were also less likely to have universal ownership of ITNs. Allocation strategies for future mass ITN distribution campaigns need to incorporate households in low socio-economic classes as a first step. More consideration should also be accorded to households with odd number household size.

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LABORATORY AND SEMI-FIELD EVALUATION OF A LONG-LASTING MICROBIAL LARVICIDE FOR MALARIA VECTOR CONTROL

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Bio-larvicides can target both insecticide resistant and outdoor biting mosquitoes however, currently available formulations have a short duration of efficacy and the need for frequent applications hence more costs involved. The study was designed to evaluate the efficacy and duration of activity of a new slow release briquet formulation of Bacillus thuringiensis israelenis (Bti) and Bacillus sphaericus (Bs) under laboratory and semi-field conditions. One and Two larvicide briquets were dissolved in separate plastic tanks to make two different Bti/Bs solutions while a non-Bti/Bs briquet was dissolved in another plastic container to make the control solution. After 24 hours, 2 liters of each solution and twenty 2nd - 3rd instar of field collected *Anopheles* larvae were added to a set up of 10 small plastic basins and placed in the insectary. The same was done for the semi-field experiment and left in the open outside the insectary. The surviving larvae and pupae in each plastic basin in both treatment and control set-ups were counted daily. The experiment was repeated on day 3, 7, 2 weeks and thereafter monthly. Results in the lab show both larvicide solutions gave high larval mortality of about 90% for the 5 months period with no larval pupation observed during the first two months. About 5%, 8% and 11% of the larvae pupated within 3, 4 and 5 months respectively for the one briquet larvicide. For the two briquet solution, about 2.5%, 4.5% and 7.5% of the larvae pupated within 3, 4 and 5 months respectively. More than 75% larval pupation was observed in all the 5 months in the control set up. In the semi-field, both the larvicide solutions gave high larval mortality for 3 months and there was no larval pupation observed in the two larvicide solutions during the first two months. In the control set up larval pupation was more than 80% for all the 4 months. This study showed that this new Bti/Bs briquet formulation is effective in killing larval mosquitoes for more than 4 months.

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DEVELOPMENT AND EVALUATION OF A NOVEL PIPE TRAP FOR OUTDOOR HOST-SEEKING MALARIA VECTOR SURVEILLANCE IN WESTERN KENYA

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Monitoring outdoor biting malaria vectors is essential to monitor residual malaria transmission and evaluate the likely success of vector control measures. Human landing catch (HLC) has been considered standard method for monitoring host-seeking vectors. However, its use is facing increasing ethical concern. CDC light trap has been used as an alternative, but it does not provide a direct estimate of human biting rate. In this study, we evaluated a novel pipe trap for surveillance of outdoor host-seeking malaria vectors. The pipe trap was made of pipe that pumps human odor from sleeping room to outdoor station and CDC light trap which was connected to the outer end of the pipe. Fan was fitted in to the pipe to enhance outflow of the odor. The sampling efficiency of the pipe trap was compared with outdoor CDC light trap (LT-outdoor) in cross-over experimental design in lowland (Ahero) and highland (Iguhu) settings of western Kenya from November 2015 to February 2016. A total of sixty trap-nights were done for each trap per site. Overall, 2,924 *Anopheles* mosquitoes were collected by pipe trap and LT-outdoor. Of these *An. arabiensis, An. funestus, An. gambiae, An. pharoensis* and *An. coustani* accounted for 18.4%, 6.2%, 0.3%, 39.8%, and 35.3%, respectively. In Ahero, pipe trap yielded significantly (Mann-Whitney p = 0.003) higher numbers of An. arabiensis (mean = 5.53, 95% CI: 3.61-7.45) than LT-outdoor (mean = 2.48, 95% CI: 1.36-3.60). Similarly, the pipe trap caught significantly (p = 0.002) higher numbers of *An. funestus* (mean = 1.65, 95% CI: 1.15-2.15) than LT-outdoor (mean = 0.78, 95% CI: 0.43-1.13). The mean numbers of other species collected did not vary significantly between the two traps. In the highland, mean numbers of An. gambiae and An. funestus was 0.1 and 0.15 in the pipe trap, and 0.05 and 0.1 in the LT-outdoor, respectively. Both traps collected mostly unfed mosquitoes, with higher proportion recorded in the pipe trap. The pipe trap attracted higher numbers of human seeking malaria vectors, suggesting its potential to replace HLC. Validation of the pipe trap for collecting outdoor human biting mosquitoes will be done by comparing against Malaise trap.

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ESTIMATING BEDNET DEMAND IN TANZANIA USING A DISCRETE CHOICE EXPERIMENT

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During the last 10 years, widespread evidence confirms that insecticide-treated bednets (ITNs) are an effective tool for decreasing malaria incidence. While this evidence has supported mass distribution of free ITNs in various sub-Saharan African countries, no current information exists regarding private household demand for ITNs. This study estimates private demand for bednets in Tanzania using an experimental approach, namely a non-hypothetical Discrete Choice Experiment (DCE). The results should prove useful to policymakers, donors, and bednet manufacturers as they explore the extent that upper and middle income households might share the cost of ITN distribution and identify preferences among potential users for various bednet attributes (texture, shape and size). Tanzania provides a suitable locale to explore these questions because the government recently implemented several nationwide distribution campaigns. The study gauges the impact of these campaigns on private demand. Moreover, the DCE provides an improved estimate of demand elasticity and willingness-to-pay for specific attributes, including the pre-treated insecticide. Existing estimates of bednet demand in Tanzania were conducted prior to the introduction of pre-treated long lasting nets (LLINs) and before the mass distribution campaigns. Consequently, the bednet market has experienced potentially significant demand and supply side shocks. The DCE method involves displaying two different nets to each participant and assigning prices to the nets. Participants then choose the net they would prefer to buy, or neither one. The DCE replicates a market environment since participants receive a small stipend, with the cost of the chosen net deducted from the stipend. DCEs are routinely used for market studies in high-income countries but this study marks their debut application to bednets. Data collection will occur during May 2016, with 800 participants spread across two rural and two urban locations.

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KNOWLEDGE, ACCEPTANCE AND WILLINGNESS TO PAY FOR INDOOR RESIDUAL SPRAY IN RURAL AND URBAN COMMUNITIES IN NIGER STATE, NIGERIA

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Malaria is the leading cause of U5 mortality (20%) and responsible for 11% maternal mortality in Nigeria. The Nigeria National Malaria Elimination Programme (NMEP) adopted In-door Residual Spray (IRS) in 2007 as one of her key strategies for reducing malaria burden in

the country. A quantitative study was conducted among households in Niger state to assess the knowledge, acceptance, and willingness to pay for IRS by households to inform nationwide IRS implementation. Respondents were selected through a multistage sampling technique. Data were collected by trained research assistants using semi-structured questionnaire, administered to 400 consenting households across six communities in two local government areas. Ethical approval for the study was granted by the Niger State Ministry of Health. Association between Willingness to pay and socio-demographic characteristics such as age, sex, education, religion and socio economic status was explored using the Chi Square Test. Result showed that only 8.4% of the respondents have ever heard of IRS therefore information on IRS was provided to all households that had never heard of it before the study. Although about 70% of the households were using LLIN to prevent malaria, 88.4% were willing to have their houses sprayed while 71% where willing to pay for IRS. Majority of the respondents were willing to pay at most N1000 (about $5) per spray while about 1.2% wanted IRS provided at no cost. Place of residence (urban, rural), status of respondent (whether household head or not), ownership of dwelling place and level of education were observed to be significantly associated with willingness to pay for IRS. The study revealed low knowledge of IRS in households in Niger state but a high level of acceptance and willingness to pay once the benefits of IRS have been provided to respondents. To facilitate acceptance of IRS, implementers should obtain consent from head of households and sensitization on the importance of IRS should be carried out. Findings from this study will guide implementation of state-wide implementation of IRS in Niger state.

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EVALUATION OF THE IMPACT OF IMPLEMENTATION OF SEASONAL MALARIA CHEMOPREVENTION ON MORBIDITY AND MORTALITY IN YOUNG CHILDREN: A QUALITATIVE STUDY IN NORTHERN GHANA

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Seasonal Malaria Chemoprevention (SMC) intervention was introduced in the Upper West Region of Ghana in July 2015. This was by the recommendation of WHO as an additional tool which has been shown to be effective, safe and feasible in preventing malaria among children under five years in areas with high seasonal malaria transmission. Community involvement is critical in controlling malaria. This may determine the effectiveness of the intervention. A study was undertaken to assess the community acceptability of this intervention in addition to other malaria control measures in reducing the burden of malaria among children under five years in the Upper West Region of Ghana. Fifty interviews and eight focus group discussions were conducted in the Lawra district where SMC intervention was introduced for the first time. Participants consisted of parents and guardians of children who received the intervention and community health workers and health volunteers. The intervention was generally acceptable to the local population who opined that it had led to a reduction in the prevalence of malaria in the district compared to previous years. An overwhelming majority of the respondents said the drug was very good because it prevented malaria in children who took it.Drug adherence was very good and there was no serious adverse event associated with drug intake. The various adverse events that occurred did not result in interruption of the treatment course. Community members and other stakeholders believe that the SMC intervention played a role in reducing the burden of malaria in the study area and wish that the program would continue so that their children would continue to benefit. Some participants called for the intervention to be introduced into the EPI and made compulsory for all children under five years. Acceptability of the intervention was very high among parents and other stakeholders in the study area. They however wanted to be counselled on the potential side effects and their management. Community acceptability of SMC is very high and can contribute to control malaria in areas where malaria transmission is seasonal.

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THE IMPACT OF INDOOR RESIDUAL SPRAYING ON THE DENSITY AND PARITY RATE OF *ANOPHELES GAMBIAE* S.L. IN OROMIA REGION - ETHIOPIA

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Entomological monitoring is an integral part of the US President's Malaria Initiative's (PMI's) vector control efforts. Data on key entomological indicators is regularly collected and used to guide vector control programs and track their impact. A non-randomized, interrupted controlled time series design was used to assess the impact of IRS on vector density and parity rate in Ethiopia. Pre-spray data was collected from one control and two intervention districts in monthly intervals from March to August 2015, while post-spray data was collected monthly from September to November 2015 from the same sites. Pyrethrum spray and human landing catch mosquito sampling methods were used to determine indoor resting density and parity rate, respectively. Before spray, the mean indoor resting density of the main vector, *Anopheles gambiae* s.l., was 4.1 (n=496 mosquitoes) and 1.03 (n=124) per house per day in the intervention and control sites, respectively. Post spray, the mean vector density dropped to 0.49 (n=59) in the intervention and to 0.73 (n=88) in the control districts. The decline in vector density was four-fold in the intervention sites as opposed to only a 30% decline in the control site. The reduction was statistically significant in the intervention (p=0.025) but not in the control (p=0.738) site. The difference noted between the two treatments might be due to the impact of IRS.At the same time, data on parity rate for *An. gambiae* s.l. was collected pre- and post-spray from the intervention and control sites. Before the spray campaign, the parity rate was 79.75% (n=553) in the intervention and 96.94% (n=193) in the control sites. After spraying, the parity rate dropped to 54.57% (n=405) in the intervention sites but increased to 100% (n=148) in the control site. The decline and increase in parity rate observed after spray as compared to before spray in the intervention and control sites, respectively, were statistically significant. (p<0.001). The change detected in parity rate might partially or totally be attributed to the impact of IRS. Further study is needed to understand if the reduction in density and parity rate has led to reduced malaria transmission.

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STRATIFICATION OF INDOOR RESIDUAL SPRAYING (IRS) IN BIOKO ISLAND: METHODOLOGY AND IMPACT

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The Bioko Island Malaria Control Project (BIMCP) uses a GIS-based Campaign Information Management System (CIMS) that uniquely identifies each household based on geographical location. 2014 spray data revealed Island wide coverage of just 57% with high refusal rates, well below the WHO recommended coverage for effectiveness. Due to this, as well as budget constraints that did not support long term-sustainability of Island-wide IRS, the BIMCP stratified households and communities targeting spraying at the most vulnerable populations only, relying on LLINs in other areas. To stratify households by vulnerability, we utilized community-level parasite prevalence and risk of importation derived from the 2014 MIS, quality of housing and 2014 IRS coverage from the CIMS. As the 2014 MIS sampling frame was based on sentinel sites, MIS values for prevalence and importation in communities not

included in the MIS sample were imputed. Based on this information, a risk score (R) and a risk-acceptability score (Ra) were derived for each community. Communities with the highest R and Ra values were selected for IRS- targeting, given the available budget, 30% of all households. Spray coverage in targeted communities was high, with an overall coverage of 80%. As such, in 2016, the BIMCP re-ran the stratification but removed previous IRS coverage as a criteria utilizing only prevalence, importation and housing quality - our measures of vulnerability. In addition, the 2015 MIS sampling frame was all communities in the Island with 20 households or more, eliminating the need to impute data. MIS data from 2014, 2015, and 2016 will be analyzed in communities that were stratified for IRS and in those that were not, to determine if the stratification of IRS and replacement of LLINs as the main control strategy in all communities resulted in a differential change in prevalence of infection across communities. An initial analysis of 2014 and 2015 data revealed prevalence of infection decreased both in communities stratified for IRS and those where LLINs alone were deployed, indicating that the withdrawal of IRS from less vulnerable communities did not lead to adverse consequences.

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COST-EFFECTIVENESS OF MALARIA CONTROL MEASURES: A CLUSTER-RANDOMIZED CONTROL TRIAL OF IRS AND ITNS IN MOZAMBIQUE

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Malaria endemic countries face challenging decisions regarding choice and financing of interventions to prevent malaria. To maintain the gains in malaria control and move toward elimination, malaria endemic countries need information on the cost effectiveness of interventions and combinations of interventions. Vector control programs, through insecticide treated nets (ITN) and indoor residual spraying (IRS), are a critical component of national malaria strategies. Yet, widespread insecticide resistance - particularly to pyrethroids, is a serious threat to program effectiveness. Additionally, important questions remain regarding the benefits and costs of IRS in the context of expanding ITN coverage. In order to inform national vector control strategies, this study will compare the cost-effectiveness of different interventions in a malaria endemic region of Mozambique by determining the cost per case averted of ITNs alone, as compared to ITNs plus IRS. Clusters will be randomized in mid-2016 to receive either IRS with the organophosphate, Actellic CS (intervention), within a context of high coverage of pyrethroid impregnated ITNs or ITN coverage only (control). Data collection is from 2016 to 2018 with baseline data available by October 2016. Changes in malaria incidence from strengthened health facility data, community parasite prevalence and net use from community surveys, entomological inoculation rates and resistance levels from entomological surveillance, and intervention costs will provide evidence on the impact of the intervention. Routine case burden data, cost data, cross-sectional prevalence surveys and entomological monitoring will be triangulated to determine overall and incremental impact and cost-effectiveness of IRS plus ITNs compared with ITNs alone. These findings will be critical to support informed-decision making to control and eliminate malaria by malaria programs in Mozambique and other high-burden countries.

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DETERMINING THE PREVALENCE AND GEOGRAPHIC DISTRIBUTION OF MIXED FUNCTION OXIDASES IN *ANOPHELES GAMBIAE* S.L. WITH PYRETHROID RESISTANCE IN RELATION TO SITE SELECTION FOR A TRIAL OF COMBINATION LLINS IN MALI

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The only long-lasting insecticidal nets (LLINs) currently under consideration for use in areas of pyrethroid resistance are LLINs treated with pyrethroids plus piperonyl-butoxide (PBO), a synergist that inhibits the activity of mixed function oxidases (MFOs) in resistant mosquitoes. This study sought to document the prevalence of MFO mechanisms in pyrethroid-resistant An. gambiae s.l. mosquitoes in different geographic areas of Mali. This information was used to identify sites for an intervention trial involving PBO-containing LLINs. *Anopheles gambiae* s.l. larvae were sampled in 12 sites in central and southern Mali from August-December 2015. Local vector populations were screened with the CDC bottle bioassay tests for permethrin and deltamethrin resistance. Bottle bioassays with PBO as a synergist were used to determine the association between reduced survival and elevated levels of MFOs. Taxonomic identification of species as well as presence of the L1014F and L1014S kdr (knock down resistance) mutations were determined by polymerase chain reaction. Based on WHO's 2013 resistance classification guidelines (mortality rate < 90%), evidence of high-frequency pyrethroid resistance was observed in all 12 test sites. Mortality increased (P97%). This suggests evidence of MFO-based metabolic resistance mechanisms involved in pyrethroid resistance. High variability was observed in resistance and the effect of PBO on mortality among sites. L1014F kdr allelic frequencies were >60% in *Anopheles coluzzii*, >40% in *An. gambiae* s.s. L1014S kdr allelic frequencies were <15% in An. coluzzii, <10% in An. gambiae s.s. This study demonstrated high variability of MFO mechanisms in wild-caught mosquitoes with pyrethroid resistance in central and southern Mali. Even though use of PBO did not restore full susceptibility, several sites were identified as potentially suitable for further evaluation of combination LLINs with PBO.

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WHY NO NETS? AN IN-DEPTH INVESTIGATION INTO THE DECREASE IN NET ACCESS ON BIOKO ISLAND AFTER BED-NET DISTRIBUTION

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From December 2014 to June 2015, the Bioko Island Malaria Control Project (BIMCP) conducted a mass-top up LLIN distribution campaign on Bioko Island, Equatorial Guinea. Despite good reported coverage (88% of households received at least 1 LLIN), results from the BIMCP's 2015 Malaria Indicator Survey (MIS) reported low levels of net ownership (69%)

and low net access (32%) approximately 7 months following distribution. While the distribution campaign ensured that one net was distributed per sleeping area, achieving an average of one net per 2 residents in 88% of households, a better understanding of the reasons behind the reduction of nets is needed to allow BIMCP to implement strategies against a decrease in net access in future campaigns. An initial analysis of MIS questions regarding net ownership indicated that a high proportion of households reported a dislike of the net (16.2%), while other households reported giving them to relatives on the mainland (12.7%). A mixed quantitative and qualitative study will be performed to determine the reasons behind the reduction in bed net access. A random sample of 150 households on Bioko Island will be selected from BIMCP's Campaign Information Management System (CIMS) and MIS databases. These databases use a GIS-based household mapping system, used to easily locate and track every household location to be sampled. A semi-structured interview will be employed to determine participant household's current net access relative to the number of nets distributed during the campaign and the number of nets reported in the subsequent MIS, what happened to the nets that were lost and the reasons why, current net usage by household member type (e.g. under-5's, pregnant women, etc.), their knowledge and attitudes acquired from the distribution teams and/or other media about LLINs, and their perceptions about the distribution process. The analysis will seek to discern what the primary reasons are for the unexpected decrease in net access. Findings will inform the BIMCP on how to reduce the LLIN loss rates, in planning for the upcoming 2017 LLIN distribution and for future continuous distribution.

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DETERMINANTS OF BED NET USE CONDITIONAL ON ACCESS IN POPULATION SURVEYS IN GHANA

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Out of 23 African countries surveyed with a Demographic and Health Survey (DHS) in the 2012-2015 period, Ghana ranks 21st in net use conditional on access (NUCA) (with 57.5%), and only Namibia and Nigeria have a larger proportion of people not using nets despite having access to a space under one. In literature, self-reported reasons for not using nets include discomfort (due to heat), disruption of sleeping arrangements, and low mosquito density. We explored if 'geographical region', 'residence' (urban or rural), 'wealth quintile', 'spraying of the dwelling with residual insecticides' (IRS), the 'humidex' in the month of survey, 'connection to electricity', the 'mean age' of the nets in the household, the 'nets : people ratio' in the household and the 'respondent heard a message on the use of nets' could explain NUCA with beta-binomial regression models that corrected for spatially correlated random effects in the Ghana 2014 DHS. Similarly, this was done with these variables (except the not collected one on messaging) for the Ghana 2011 Multiple Indicator Cluster Survey (MICS), with as additional variables 'ethnicity', 'religion' and an estimate of 'vector density in the month of survey'. In the DHS, 'wealth quintile', 'electricity', 'residence', 'messaging', 'humidex' and 'IRS' (in order of decreasing importance) individually improved model fit, as assessed by the deviance information criterion (DIC), compared to a null model without covariates, and also all these variables combined in one model improved the DIC over simpler models. In the MICS, the variables 'region', 'net age', and 'IRS' improved the fit when individually added to the null model. 'Net age' improved the fit when included into a model with 'region' as covariate. Differences in results between 2011 and 2014 are possibly due to the 2011 MICS being held in the midst of a mass distribution campaign, with 4 regions (partly) completed and 6 not yet begun. Surprisingly, the 'nets : people ratio' (the higher the ratio, the less need for sharing nets and possibly disrupting sleeping arrangements) did not explain NUCA and the humidex (a measure of comfort due to heat) only did so in the 2014 DHS.

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JOINT DISTRIBUTION OF ACUTE RESPIRATORY INFECTION, DIARRHEA AND STUNTING AMONG CHILDREN UNDER THE AGE OF FIVE YEARS IN SOMALIA

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The aim of this study was to assess the spatial co-occurrence of acute respiratory infections (ARI), diarrhoea and stunting among children the age of five years in Somalia. Data were obtained from routine bi-annually nutritional surveys conducted by the Food and Agriculture Organization (FAO) from 2007 - 2010. A Bayesian hierarchical shared component model was fitted to the spatial components of the three health conditions concurrently. Risk maps of the common spatial effects at 1 x 1 km resolution were derived. The empirical correlations of the proportion were 0.37, 0.63 and 0.66 for ARI and stunting; diarrhoea and stunting and ARI and diarrhoea respectively. Spatially, the posterior residual effects ranged from 0.04 to 18, 0.19 to 5.39 and 0.07 to 8.16 for shared component between ARI and stunting; diarrhoea and stunting; and ARI and diarrhoea respectively. This analysis shows clear spatial shared component between ARI, diarrhoea and stunting in Somalia with the southern part of the country experiencing a higher than usual rate of these conditions. Intervention aimed at reducing the rates of these three health conditions should focus on the common risk factors particularly in the South in Somalia.

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SHANCHOL, THE ORAL CHOLERA VACCINE IS SAFE AND IMMUNOGENIC WHEN STORED AT ELEVATED TEMPERATURES IN BANGLADESHI PARTICIPANTS

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Immunization against cholera is recognized as a major intervention for control of the disease, however the requirement for a cold chain has limited its use in resource poor settings.The aim was to determine the safety and immunogenicity of Shanchol after stored at temperature upto 42°C. The study conducted in adult participants in Dhaka, Bangladesh. The vaccine kept under standard (Group A; 2-8°C) and at three elevated temperatures (Group B; 25°C, Group C; 37°C, Group D; 42°C) for 14 days. Vibriocidal and LPS antibody responses were determined at baseline and 7 days after each dose. 580 participants; 145 in each group (A=2-8 °C ; B=25 °C; C=37 °C and D= 42°C) were vaccinated. Only 17 mild adverse events, which did not differ between groups. Increases of vibriocidal responses observed at day 7 and 21 ($P<0.001$) compared to day 0 in all groups. Over four-fold increases to *V. cholerae* O1 Ogawa were observed at day 7 and/or day 21 after vaccination in at all groups, with responder rates of; 76%, 80% , 69% and 74% in Groups A, B, C, and D ($p=0.240$). Significant vibriocidal antibodies to *V. cholerae* O1 Inaba and O139, and LPS- Ogawa and Inaba antibody responses also observed after each dose ($p<0.001$); with comparable seroconversion rates between Group A and Groups B, C, and D. Interpretation: This is the first report of the testing

the temperature stability of Shanchol in people. Shanchol is safe and immunogenic in endemic settings even when kept outside the cold chain. Further study should conduct to assess the impact in younger age group.

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HOST AND ENVIRONMENTAL CORRELATES OF MULTI-DRUG RESISTANCE IN KENYAN CHILDREN WITH ACUTE BACTERIAL DIARRHEA

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Bacterial diarrhea results in significant morbidity and mortality in children in sub-Saharan Africa. Antibiotic treatment may be important in this population but is limited by concerns about antibiotic resistance. Stool/rectal swab samples of children aged 6 mos - 15 yrs presenting with acute diarrhea in western Kenya were cultured for bacterial pathogens. HIV uninfected children with identified *Shigella* or *Salmonella* species, or enteropathogenic [EPEC], enterotoxigenic [ETEC], enteroaggregative [EAEC], or enteroinvasive *Escherichia coli* [EIEC] were included in this substudy. Resistance to ampicillin, ceftriaxone, ciprofloxacin, cotrimoxazole, and tetracycline was determined using MicroScan Walkaway40 Plus. To evaluate correlates of multi-drug resistance (MDR [resistance to ≥ 3 classes of antibiotics]), we used multivariable log-binomial regression to estimate prevalence ratios (PRs) and 95% confidence intervals (CIs). Of 336 children in the analysis, median age was 19 mos (interquartile range: 10-39 mos), 38% used unimproved sanitation facilities and 10% were HIV-exposed. Resistance to cotrimoxazole (96%) was most common among all pathogens, followed by ampicillin (81%) and tetracycline (77%). Phenotypic MDR was identified in 11% of children; and in 38% of *Shigella*, 40% of *Salmonella*, 76% of EPEC, 54% of ETEC, 78% of EAEC, and 77% of EIEC isolates. Children 6-24 mos were more likely to have MDR infections identified than those 24-59 mos (aPR = 1.56 [95% CI: 1.25, 1.92]). Children whose caregivers used a shared pit latrine or who openly defecated were more likely to have MDR (aPR = 1.71 [95% CI: 1.02, 2.86]) than those with flush or unshared toilets. Duration of exclusive breastfeeding, malnutrition, maternal HIV, water source, and hand washing were not associated with MDR infections in this study. Young age and unimproved sanitation may be associated with MDR as a result of greater exposure to fecal contamination. Community antibiotic use may select for resistant enteric bacteria in settings with poor sanitation by eliminating susceptible bacteria before excretion and increasing inter-species transfer of resistant plasmids.

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GENOTYPIC IDENTIFICATION OF AMPC β-LACTAMASES PRODUCTION IN DIARRHOEAGENIC E.COLI FROM CHILDREN UNDER FIVE AND MOLECULAR DOCKING OF THEIR PROTEINS

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AmpC beta-lactamases are bacterial enzymes that hydrolyse 3rd generation extended spectrum cephalosporins and cephamycins engendering resistance to these categories of antibiotic. AmpC β-lactamase expression can increase in the nosocomial pathogens, triggered by exposure to antibiotics and β-lactamase inhibitors with the β-lactam function. Therefore, AmpC β-lactamase is an important target for developing novel effective antibacterial therapies. The objective of this study was to evaluate the Real Time PCR as a rapid diagnostic tool for simultaneous detection of AmpC beta-lactamase producing E.coli and in silico determination of docking sites of AmpC proteins.During one year period from July 2012 to July 2013, 120 stool samples were collected, including 80 diarrheagenic E.coli and 40 controls from children in University College of Medical Sciences and Guru Teg Bahadur Hospital, East Delhi. E.coli was diagnosed for AmpC beta lactamase production using conventional phenotypic tests. DNA extraction was done, and extracted DNA was used as a template for Real Time PCR. Bioinformatics tools were used for molecular docking. Real time PCR detected target genes of AmpC beta lactamase in 18.75% and 22.5% in cases and controls respectively. Chi square test and Fisher's exact test were used to determine statistical significance of data. Real time PCR assay will save time and help investigators to explore the role of multidrug resistant *E. coli*. Active binding sites will be useful in synthesis of new drugs. Modeling and docking studies may provide useful insights for developing new antibiotic drugs to minimize multidrug resistance.

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CAMPYLOBACTER SPECIES, CAPSULE TYPES AND VIRULENCE FACTORS CIRCULATING AMONG CHILDREN IN EGYPT

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Campylobacter is one of the most frequently isolated bacterial pathogens in children with diarrhea. There are 47 recognized Penner serotypes of *Campylobacter jejuni*, represented by 35 capsule types. The capsule serves as a target for vaccine development. *Campylobacter* can also harbor the type-6 secretion system (T6SS), which is a known virulence factor among Gram negative pathogens. Data are lacking on the distribution of *C. jejuni* capsule types in most developing countries. The prevalence of the T6SS within *Campylobacter* species and capsule types has not previously been reported. The US Naval Medical Research Unit #3, based in Cairo, Egypt conducted three prospective diarrheal studies in Abu Homos, Egypt from 1995-2000. In total, 531 *Campylobacter* spp. isolates were obtained from 397 children. Each isolate was re-grown and DNA was extracted for speciation, capsule typing and hcp gene presence, a marker for T6SS. Of the isolates, only 522 and 501 were respectively available for capsule and T6SS analysis; 60.9% (318) were *C. jejuni*, 37.9% (198) were *C. coli* and 1.1% (6) were unable to be speciated. Capsule types were identified in 88.4% (281) of the *C. jejuni* isolates; HS-2 and HS-3 were the most prevalent (both 11.9%). The hcp gene was found in 57.5% (172) of *C. jejuni* isolates compared to 19.4% (38) of *C. coli* isolates ($p<0.001$) and 83.3% (5) of the unspeciated isolates ($p<0.578$). The presence of the hcp gene among *C. jejuni* capsule types varied significantly (0-100%). This study provides epidemiological data regarding the distribution of circulating *C. jejuni* capsule types. Within this cohort, eight capsule types together accounted for 72% of *C. jejuni* infections. The heterogeneity of *C. jejuni* serotypes is higher than previously reported, although prior studies of serotype distribution within Africa are limited and have not focused on pediatric infections. Our data suggests that the T6SS is more commonly found among *C. jejuni* isolates compared to *C. coli*. We also highlight significant gaps that persist. Further studies are needed to determine whether specific capsule types or presence of the T6SS are associated with more severe clinical illness.

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ENTEROPATHOGENS DISTRIBUTION AND BURDEN WITHIN ORAL CHOLERA VACCINE RECIPIENTS IN SOUTH SUDAN

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The efficacy of oral cholera vaccine (OCV) is poorer in younger children than adults and there is great heterogeneity in immunogenicity within and between different sub-populations. Multiple oral vaccines have shown decreased efficacy in the setting of concurrent enteric infections. The objective of this study was to explore the distribution of enteropathogens within stool of recipients of OCV and the relationship between pathogen carriage and immunogenicity. As part of a mass vaccination campaign in an internally displaced person's camp in South Sudan, we obtained paired stool and serum samples from a subset of recipients who received 2 doses of the OCV Shanchol 14 days apart. We used the vibriocidal assay to determine titers just before the first dose (day 0) then 14 and 28 days after. We extracted nucleic acid from stool samples and performed multiplex real-time PCR on 18 bacterial, viral and protozoal targets. Stool from a total of 89 subjects were examined, 13 (15%) of which reported diarrhea the week prior to vaccination. Overall, 39 of 89 (44%) subjects had at least one pathogen identified in stool, with 19 (21%) having two or more identified. Among those aged 1-5 years (young children, n=8), 6-17 years (older children, n=26) and 18-60 years (adults, n=55), we found stools to be positive for at least one pathogen in 50%, 50%, and 38% of subjects, respectively. The most commonly identified pathogens were Shigella spp. (13% of subjects), D fragilis (12%), norovirus (12%), and STEC (9%). 32 of 46 (70%) subjects with vibriocidal data available seroconverted. We did not find any significant differences in the presence of co-infection, type of co-infection or extent of pathogen burden between those who did and did not seroconvert. We demonstrate that a large proportion of OCV recipients in an internally displaced person camp in South Sudan had asymptomatic carriage of enteropathogens. While these preliminary analyses suggest that these co-infections may not be associated with immune responses to OCV, we are in the process of completing analysis of additional pathogens, including helminthic targets, and final data will be available by time of presentation.

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EFFECT OF *HELICOBACTER PYLORI* INFECTION ON GROWTH TRAJECTORIES IN YOUNG ETHIOPIAN CHILDREN: A LONGITUDINAL STUDY

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Helicobacter pylori infection has been associated with early childhood growth impairment in high and middle-income countries; however, few studies have examined this relationship within low-income countries or used a longitudinal design. We examined the possible effects of *H. pylori* infection on growth trajectories in a cohort of young Ethiopian children. In 2011/12, 856 children (85.1% of the 1006 original singletons in a population-based birth cohort) were followed up at age six-and-a-half. An interviewer-led questionnaire administered to mothers provided information on demographic and lifestyle variables. Height and weight were measured twice, and the average of the two measurements was used. Exposure to *H. pylori* infection was assessed using a rapid *H. pylori* stool antigen test. The independent associations of positive *H. pylori* infection status (measured at ages 3 and 6.5 years) with baseline height and weight (age 3) and height and weight growth trajectory (from age 3 to 6.5 years) were modeled using Hierarchical Linear Models. At baseline (age 3), children's mean height was 85.7 cm and their mean weight was 11.9 kg. They gained height at a mean rate of 8.7 cm/year, and weight at a mean rate of 1.76 kg/year. *H. pylori* infection was associated with lower baseline measurements and linear height trajectory (β=-0.74cm, and -0.79cm/year, respectively), after controlling for demographics and markers of socio-economic status. However, the positive coefficient was associated with quadratic growth in height among *H. pylori* infected children (β=0.28, 95% CI, 0.07 to 0.49, p<0.01), and indicated increase in height trajectory as the child increased in age. In conclusion, our findings add to the growing body of evidence supporting that *H. pylori* infection is inversely associated with childhood growth trajectory, after controlling for a range of factors associated with reduced growth and *H. pylori* status.

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ANTIMICROBIAL RESISTANCE PATTERNS AMONG INTERMEDIATE - TO LONG-TERM TRAVELERS TO CUSCO, PERU, WITH DIARRHEA

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In order to characterize antimicrobial resistance among travelers with diarrhea in Cusco, Peru, we prospectively collected stool from students older than 18 years of age presenting consecutively to the Amanta Spanish School's physician with diarrhea between June 2003 and July 2010. We performed antibiotic susceptibility testing using the disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2011). Reduced susceptibility to ampicillin and first-generation cephalosporins was common among all isolates, except Shigella sonnei. Non-*Campylobacter* isolates were nearly uniformly susceptible to ceftriaxone. *Campylobacter coli* and *C. jejuni* isolates had reduced susceptibility to erythromycin and both quinolones, naldixic acid and ciprofloxacin, whereas susceptibility to azithromycin was 100% for both species and susceptibility to sulfamethoxazole-trimethoprim 100% and 94%, respectively, for both species. For the *E. coli* enteropathogens, ETEC was the most common isolate and was highly susceptible to amoxicillin-clavulanic acid (89%), ceftriaxone (100%), azithromycin (79%), and ciprofloxacin (93%), but showed greatly reduced susceptibility to cefazolin (64%), erythromycin (4%), sulfamethoxazole-trimethoprim (61%), and tetracycline (61%). Susceptibility trends for EAEC were similar to ETEC. Of the Shigella sp., S. sonnei strains were more common than S. flexneri and notable differences in susceptibility between the two species were found. Shigella sonnei was more susceptible to ampicillin (100%), cefazolin (100%), and sulfamethoxazole-trimethoprim (100%) than S. flexneri, but less susceptible to azithromycin (56%). Ciprofloxacin, tetracycline, and ceftriaxone susceptibility was 100% for both S. sonnei and S. flexneri. These results suggest that azithromycin is the most appropriate empiric treatment for travelers' diarrhea in this region, as it will effectively treat ETEC, Shigella, and fluoroquinolone-resistant *Campylobacter*-associated diarrhea, whereas ciprofloxacin would only effectively treat ETEC and Shigella.

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LETHALITY OF FIRST CONTACT DYSENTERY EPIDEMICS ON PACIFIC ISLANDS

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Infectious diseases de-populated many isolated Pacific islands when they were first exposed to global pathogen circulation from the 18th century. Although the mortality was great, the lack of medical observers makes determination of what happened during these historical epidemics largely speculative. Bacillary dysentery caused by Shigella is the most likely infection causing some of the most lethal island epidemics. The fragmentary historical record is reviewed in order to gain insight into the possible causes of the extreme lethality that was observed during first contact epidemics in the Pacific. Subacute dysentery occurred following first contact measles epidemics but not in subsequent smaller measles outbreaks. Immune aspects of the early dysentery epidemics and post-measles infection resulting in subacute inflammatory enteric disease suggest that epidemiologic isolation was the major lethality risk factor on Pacific islands in the 19th century. Other possible risk factors include HLA homogeneity from a founder effect and pathogen-induced derangement of immune tolerance to gut flora. If this analysis is correct, then Pacific Islands are currently at no greater risk of emerging disease epidemics than other developing countries despite their dark history.

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PLASMA AND MEMORY B CELL RESPONSES TARGETING O-SPECIFIC POLYSACCHARIDE (OSP) ARE ASSOCIATED WITH PROTECTION AGAINST *VIBRIO CHOLERAE* O1 INFECTION AMONG HOUSEHOLD CONTACTS OF CHOLERA PATIENTS IN BANGLADESH

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Vibrio cholerae is a noninvasive intestinal pathogen, and the cause of cholera, a severe dehydrating illness in humans. The mediators of protection against cholera are currently unknown. However, we have previously shown that IgG memory B cell responses targeting lipopolysaccharide (LPS) of *V. cholerae* O1 correlate with protection against *V. cholerae* O1 infection among household contacts of cholera patients. Protection against cholera is serogroup specific, and serogroup specificity is defined by the O-specific polysaccharide (OSP) component of LPS. Therefore, we prospectively followed household contacts of cholera patients to determine whether OSP-specific immune responses present at the time of enrollment are associated with protection against *V. cholerae* infection over a 30 day period. Two hundred forty two household contacts of 150 index patients with cholera were enrolled. The presence of OSP-specific IgG memory B cell responses in peripheral blood on study entry was associated with a decrease in the risk of infection in household contacts (43% reduction; $p = 0.048$). No protection was associated with cholera toxin B subunit (CtxB)-specific memory B cell responses. In addition, the presence of OSP-specific plasma IgA, IgM, and IgG antibody responses on study entry was also associated with protection among household contacts. These results suggest that immune responses that target OSP, both in plasma and memory responses, may be important in mediating protection against infection with *V. cholerae* O1.

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ROTAVIRUS VACCINE TAKES SEASONAL SIGNATURE OF CHILDHOOD DIARRHEA BACK TO PRE-SANITATION ERA IN BRAZIL (AND THAT IS A GOOD THING!)

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Diarrhea mortality and morbidity have not only declined substantially in developing nations, but also switched from a summer-bacterial to a predominantly winter-viral seasonal pattern. However, little is known about the effect of the rotavirus vaccine introduction on seasonal patterns of diarrheal disease. Here we examined the long-term evolution of diarrheal morbidity and mortality risks across age and geography in Brazil, including the effect of rotavirus immunization (introduced in 2006) and other development indicators. Nationwide mortality (1979 - 2013) and hospitalization (1998 - 2013) data were obtained from the Brazilian Ministry of Health. Secular trends and seasonality were inspected for each Brazilian state and age group using the software Epipoi. For most states, the primary peak in mortality risk among children under 5 years occurred from December to April (summer/early autumn) from 1979 - 1988, then switched to the period between June and October (winter) by 2000 - 2005 (just prior to the 2006 implementation of the rotavirus vaccine for children under 1), and next shifted back to summer/early autumn by 2007 - 2013. A similar pattern was observed for hospitalizations. In contrast, the risk of diarrhea-related death among older children (5 to 19 years) and adults (≥ 20 years) did not have a well-defined seasonality or spatial pattern until 2007, when a trend towards summer/autumn peaks of mortality was observable. Hospitalizations for these age groups tended to peak in summer/early autumn throughout the period. Rotavirus vaccination policies were not only associated with a change in the risk of death, but also with a shift in the timing of peaks in risk in children under 5 years, which is reminiscent of the summer-diarrhea period. Additionally, young children were shown to have distinct annual disease patterns compared to other age groups, suggesting a different etiology of disease.

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REACTIVE VACCINATION IN NSANJE, MALAWI USING AN ORAL CHOLERA VACCINE

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Malawi experiences regular cholera outbreaks, particularly in the southernmost district of Nsanje (population: 292,083 people) where Lake Malawi drains, causing frequent inundations. Flooding occurred in January 2015 and the President of the Republic of Malawi declared a state of disaster. One month later the first cholera case was reported, marking the beginning of an outbreak, during which Nsanje was one of the most heavily affected districts. At that time, the International Vaccine Institute (IVI) had planned to conduct a preemptive oral cholera vaccination (OCV) campaign in Nsanje in collaboration with the Malawi Ministry of Health (MOH), targeting 50,000 people with a two-dose regimen of Shancol® (Shanta Biotechnics, Hyderabad, India). As the outbreak began to spread, the Malawian MOH addressed the International Coordinating Group (ICG) and requested additional cholera vaccine doses from the WHO emergency OCV global stockpile to equip a public health response. IVI-procured vaccines were repurposed for this activity. A multi-organizational task force was put in place to plan and deliver cholera vaccination. 160,482 people including 70,000 internally displaced people were targeted for vaccination; overall 156,592 (97.6%) received the first dose, of which 108,440 (67.6%) received both scheduled doses. Here we report on the experiences in planning the activities, the outcome and lessons learnt

of the vaccination campaign as well as a comparison of this outbreak response to other reactive oral cholera vaccine (OCV) campaigns in sub-Saharan Africa conducted to date.

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HUMAN IMMUNE RESPONSES AGAINST ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) YGHJ MUCINASE, A PROMISING NEW ETEC VACCINE ANTIGEN

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The human enterotoxigenic *Escherichia coli* (ETEC) vaccine development effort has mainly been focused on inducing protective immunity by immunization with the ETEC colonization factor (CF) and heat-labile toxin antigens. But the main contributors to childhood ETEC diarrhea are heat-stable toxin-producing ETEC strains, of which about one third lack any known CFs. Identifying additional broad-coverage ETEC vaccine antigens is therefore important. YghJ, a chromosomally encoded mucinase common to most *E. coli*, and present in close to 100% of all ETEC, is a promising alternative. YghJ helps ETEC reach the small intestinal cell wall by breaking down the protective mucus barrier, and ETEC seem to secrete substantially more YghJ than other *E. coli*. We aim to describe immune responses to YghJ in human volunteers who have been experimentally infected with wild-type ETEC strains. Saliva, serum and intestinal lavage were obtained from adult human volunteers before (d0) and 10 days after (d10) experimental infection with wild-type strains TW10598 (O6:H16, CS2, CS3, CS21, STh, LT; 30 volunteers) and TW10722 (O11 5:H5, CS5, CS6, STh; 9 volunteers). T-cell responses were measured by flow cytometry gating for activated CD4 T cells expressing CD25 and CD134 after 2 days culture with YghJ. Antibody responses were measured by immunoassays where beads had been coated with purified recombinant YghJ. In preliminary analyses we found serum IgA antibody responses towards YghJ increasing significantly from d0 to d10; mean fluorescence 2625 to 14187, p=0.043, while IgG responses did not increase significantly. T cell responses increased significantly from 0.73 to 1.37 percent activated YghJ-specific CD4 T cells out of all CD4 T cells, p=0.008. In general there was higher background to YghJ than to more distinct ETEC proteins like the CS5 colonization factor, suggesting previous exposures to this protein. We have found that human volunteers experimentally infected with ETEC induce an anti-YghJ IgA antibody response, as well as T-cell responses. Further investigation into YghJ responses in saliva and intestinal lavage fluids are ongoing and results will be presented.

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OBSERVATIONS ON THE NATURAL HISTORY OF CYSTIC ECHINOCOCCOSIS IN UNTREATED AND ALBENDAZOLE-TREATED PATIENTS

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The natural history of cystic echinococcosis (CE), a complex, neglected, and arcane zoonotic disease with a global distribution, is still being elucidated. This study examined the natural history and albendazole-induced changes of CE, and whether the standardized WHO ultrasound (US) classification reflects these changes. Patient data were collected during mass US screening surveys in endemic regions amongst transhumant populations, the Turkana of Kenya, and the Berber of the Mid-Atlas Mountain region of Morocco. Cysts were categorized using the WHO US classification (CL, and CE1 to CE5). Patient records occurring prior to the receipt of treatment, and after the administration of albendazole, were selected. 852 paired before/after observations of 360 cysts (10 CL, 140 CE1, 7 CE2A, 14 CE2B, 5 CE3A, 144 CE3B, 38 CE4, 2 CE5) from 257 patients (241 Turkana, 16 Berber) who went untreated for up to 9789 days (mean 600 days, median 215 days) were analyzed using a McNemar-Bowker $\chi 2$ test for symmetry, which achieved significance ($p < .0005$). Next expected observations of cyst type exhibited no instances of regression to earlier types, only progression. A McNemar-Bowker $\chi 2$ test of 1414 paired before/after observations of 288 cysts (93 CE1, 6 CE2A, 6 CE2B, 5 CE3A, 158 CE3B, 19 CE4, 1 CE5) from 157 Turkana patients treated with albendazole also achieved significance ($p < .0005$). Regression from the CE4 to CE3B type occurred in a small percentage (2.05%) of these observations. The significant finding of asymmetry for the McNemar-Bowker test and the absence of instances of cyst regression for the natural history group re-affirms that the standardized WHO US classification is reflective of the natural history of CE, and more reflective of the natural history of CE than earlier classifications. Similarly, the significant McNemar-Bowker test for the albendazole-treated group demonstrates that the WHO classification also reflects the albendazole-induced changes in cysts. The regression of CE4 to CE3B in a few of the albendazole-treated cysts may reflect the stability of the CE3B cyst type.

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G1 STRAIN OF *ECHINOCOCCUS GRANULOSUS* IN SUDANESE PATIENTS: A THREATENING ALARM

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Cystic echinococcosis is a zoonosis caused by the cestodes *Echinococcus* spp. Its life cycle involves dogs and other canids as definitive hosts for the intestinal tapeworm, as well as domestic and wild ungulates including human as intermediate hosts for the tissue -invading metacestode (larval) stage. It was previously suggested that few data are available on the frequency of CE in humans in Sudan compared with published data on animal cystic echinococcosis. Few recent reports mentioned a prevalence of about 0.33% in central Sudan and sporadic cases in different areas in the Sudan. Recently, cystic echinococcosis is having an increased attention in Sudan as it is now more frequent having patients suffering from either respiratory or abdominal symptoms found to be associated with the presence of cyst or cysts in a vital organ associated with either of these systems. This emerging situation necessities studying the factors which have an impact on the increasing frequency of such an important disease in a country like Sudan suffering from tribal un restless and poverty. One of these factors was thought to be that 100% of cyst material obtained from human were genetically identified as *Echinococcus* canadensis G6. The infectivity of this taxon for humans was in doubt for many years, and even today the number of confirmed human cases is small and scattered all over the world and that was considered as the main reason of having a rather rare disease in Sudan despite all epidemiological conditions for autochthonous transmission of CE. From the other hand, *E. granulosus* G1 which is highly prevalent in regions known to be endemic for the disease was thought to be extremely rare in most of Sudan, as there was only one record from a dog in the central part of the country. However characterization of genetic materials recently obtained from human patients showed that this strain is present in human. A situation alarming the need of further studies to investigate to what extent is it present, population at risk and preventive measure. Hence this study represents the first study confirming the presence of G1 in patients in Sudan, knocking an alarm and discussing a preventive policy.

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CDNA LIBRARY FROM *TAENIA SOLIUM* RACEMOSE CYST: A NOVEL TOOL FOR TRANSCRIPTOMICS IN NEUROCYSTICERCOSIS

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Human neurocysticercosis (NCC), caused by the larval stage or cysts of the flatworm *Taenia solium*, is the most frequent parasitic disease of the brain in humans and is endemic in many developing countries. The murine model of NCC using *T. crassiceps* has advanced our understanding of immunopathology. However most molecular mechanisms involved in the host-pathogen interaction remain unknown. The best studied form of NCC is parenchymal disease in which cysts are lodged in the parenchymal tissue. Cysts that develop in subarachnoid spaces can grow into very large multivesicular structures commonly named "racemose cysts", which appear to differ in many characteristics from parenchymal cysts, with an aggressive course of infection that is difficult to treat, and has a high mortality rate. Investigation of *T. solium* at the molecular level is limited to detection and identification; a characterization of genes involved in regulation of growth, proliferation, and virulence has not been undertaken to date. The identification of the genes involved in survival and proliferation of racemose parasites would advance our understanding of this aberrant and malignant form of the disease. To this end, we isolated total RNA from a human *T. solium* racemose cyst, obtained at surgery, synthesized cDNA, generated double strand cDNA (ds cDNA) by long distance PCR, purified the ds cDNA using CHROMA SPIN columns, and cloned it into pSMART2IFD (In-Fusion, Clontech). Electrocompetent *E. coli* TG1 cells were transformed with these plasmids and a cDNA library was generated successfully. This library, the first generated from a *T. solium* racemose cyst, will allow systematic studies of the development and biology of the parasite and will aid in the search for novel targets for diagnostic and anthelmintic drugs. Our immediate aim is amplification and sequencing of full-length cDNA fragments by long distance PCR for cloning and identification of genes with significant roles in proliferation and cyst growth, and to determine their role in the development of the racemose parasite. This cDNA library promises to serve as a tool for detailed studies of the biology of *T. solium*.

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RISK FACTORS FOR REFRACTORY EPILEPSY DEVELOPMENT IN NEUROCYSTICERCOSIS

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Epilepsy is a major public health problem linked to increased mortality. The helminth infection Neurocysticercosis (NCC) is the number one cause of adult epilepsy worldwide; 70-80% of people with NCC experience seizures that can develop into treatment-refractory epilepsy (TRE). Early intervention before recurrent seizures establish abnormal electrical circuits in the brain could impact disease progression, but in order to do so predictors of TRE in NCC patients need to be identified. To identify such predictors, we conducted a retrospective review of 39 randomly selected subjects from a large clinical trial of patients with NCC and seizures. These subjects presented a mean of 14.9 months following their first seizure (SD, 64.3 months), most commonly had partial seizures (29/39 subjects, 74%), and had a mean of 3.3 viable or degenerating lesions due to NCC (range 1-21, SD 6.6). Eleven subjects went on to develop TRE (defined as seizures > 2 months after treatment for NCC despite anticonvulsant therapy) and 28 did not. We examined a number of clinical factors in these patients in order to identify variables associated with TRE. The primary risk factor identified was a strong and sustained inflammatory response to the parasite. This was demonstrated by a significantly increased number of bands seen on the initial NCC western blot in subjects who developed TRE (GM 5.8 bands) compared to those who did not (GM 3.8 bands, $p<.05$). As further evidence that ongoing brain inflammation is associated with epilepsy risk there was an increased (though not statistically significant) percentage of subjects with continued edema on follow-up imaging 6-12 months following treatment in those with TRE (2/6, 33%) compared to those without (2/18, 11%, $p=.6$); the total volume of edema at follow-up was also increased in those who developed epilepsy (22.7 cm^3) compared to those who did not (1.8 cm^3, $p=.2$). These findings provide a compelling rationale for further exploring signs and symptoms of ongoing inflammation as predictors of epilepsy development in NCC patients; this knowledge could aid in the development of targeted antiepileptogenic treatments.

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A CROSS-SECTIONAL ABATTOIR STUDY ON *TAENIA HYDATIGENA* INFECTIONS IN PIGS IN BURKINA FASO

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Taenia hydatigena is a non-zoonotic tapeworm transmitted between dogs, and ruminants or pigs. While *T. hydatigena* does not cause clinical signs in pigs, its presence impacts the validity of serological tests used for the diagnosis of current porcine *T. solium* infection through cross-reactions. Estimating *T. hydatigena* prevalence is crucial to validly interpreting *T. solium* serological results. The only five studies published in Africa, including 888 pigs, reported prevalences from 1.4 to 6.6%. We performed a cross-sectional study in Koudougou, Burkina Faso, to estimate the prevalence of *T. hydatigena* in slaughtered pigs. Pigs came from nearby villages practicing traditional breeding with free roaming. Over 35 days, 452 pigs were carefully inspected post-mortem for the presence of abdominal *T. hydatigena* cysticerci including the liver. In addition, meat inspectors routinely inspected the carcasses for *T. solium* cysticerci and blood samples were taken for B158/B60 Ag-ELISA examination. Cysticerci were stored in ethanol for molecular analysis by PCR-RFLP. *T. hydatigena* cysticerci were found in liver, abdomen and both in 27, 12 and one pigs, respectively. Meat inspectors found seven carcasses infected with *T. solium* cysticerci; molecular analysis confirmed six of these, one was identified as *T. hydatigena*. Seven cysts found in the liver were molecularly identified as *T. solium*. Overall, 41 carcasses (9.1%) were found infected with *T. hydatigena* and 14 (3.1%) with *T. solium*; two pigs were infected with both *Taenia* spp. 219 pigs (48.5%) were positive to the Ag-ELISA of which 23/41 (56%) and 9/14 (64%) were of pigs with *T. hydatigena* and *T. solium* cysts, respectively. The relatively high prevalence of *T. hydatigena* in this study suggests that estimates of current *T. solium* infection prevalence should be adjusted for the presence of *T. hydatigena*. Adjustment values can be estimated through post-mortem examination. *T. solium* prevalence derived from meat inspection is invalid as routine meat inspection has a low sensitivity, and pigs with tongue cysts are mostly diverted to the unofficial slaughter circuit.

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EFFICACY OF A SINGLE-DOSE OF OXFENDAZOLE AT THREE DIFFERENT FORMULATIONS AGAINST THE LARVAL STAGE OF *TAENIA SOLIUM* IN NATURALLY INFECTED PIGS

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Oxfendazole (OFZ) is the drug of choice against porcine cysticercosis. This drug is commercialized as a broad-spectrum antiparasitic for farm animals. However, its high cost limits its use by many health programs in low-income countries, so local formulations of OFZ could be an alternative for its implementation This study evaluated the efficacy of three different formulations of OFZ against porcine cysticercosis in a randomized controlled trial. Twenty-eight pigs naturally infected pigs with *Taenia solium* cysts were distributed in the following treatments: OFZ in a commercial formulation (Synanthic 9.06% Fort Dodge®, Mexico), OFZ in two experimental formulations (22.5% and 10.9%) and an untreated group. Experimental formulations were prepared in the Laboratory of Pharmacology at the National University of San Marcos University in Lima, Peru using OFZ p.a (Spectrum Laboratory Products Inc, Gardena, CA). OFZ was given to pigs at a single oral dose of 30 mg/kg under experimental conditions. Necropsies were performed at day 60 post-treatment and the carcasses were examined for the presence of cysts in muscle samples and brains. OFZ efficacy against cysts in muscle was 100% for the three treatment groups (viable cysts were not observed) compared to the untreated group ($p<0.001$). OFZ efficacy in brain was variable and non-statistical differences in the proportion of pigs with viable cysts in brains among treatments were observed. Although some differences in the plasmatic maximum concentration and the time to peak concentration in plasma after oral administration of OFZ at 30mg/kg in pigs were reported previously using the same formulations, we found no differences in the efficacy among the tested OFZ formulations. Since pigs are is a vital component in the transmission chain of *T. solium*, a cost-effective treatment in endemic areas should greatly help to eliminate the source of infection in humans. From a practical point of view, our results provide evidence that local formulation of OFZ at high concentrations (thus requiring lower volumes to be given to pigs) can be cheaper and efficient alternatives for use in the control of *T. solium*/cysticercosis.

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NEUROCYSTICERCOSIS AND EPILEPSY AMONG PEOPLE LIVING NEARBY PIGS HEAVILY-INFECTED WITH CYSTICERCOSIS IN RURAL ENDEMIC PERU

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Neurocysticercosis (NCC) causes substantial neurologic morbidity in endemic regions around the world. Ring strategy for control involves focusing treatment resources for taeniasis and porcine cysticercosis within clusters (rings) of households surrounding a heavily-infected pig. This strategy is based on the assumption that risk of parasite transmission is greatest within the rings. At the end of a community trial of ring strategy in northern Peru, we offered neurologic screening to all participants who resided within an identified ring. Our objective was to describe the prevalence of NCC and epilepsy in these rings. We offered screening to 576 ring residents by applying a 9 question seizure survey. Our study physician evaluated positive respondents to confirm the diagnosis using International League Against Epilepsy criteria. Those with confirmed epilepsy were offered a non-contrast CT of the head. We also tested sera using EITB LLGP to detect anti-cysticercus antibodies and ELISA B60/B158 to detect cysticercosis antigens. Those with strongly positive ELISA (ODR>=3) were offered a non-contrast MRI of the brain. 527 people completed seizure screening and 114 (21.6%) were positive. The physician evaluated 108, of which 35 had confirmed seizures and 16 had epilepsy (lifetime prevalence 30 per 1000). 12 with epilepsy accepted CT scan and 5 (41.7%) had parenchymal calcifications. None had viable cysts. Of the 514 that provided a blood sample, 241 (46.9%) were seropositive by EITB, 39 (7.6%) were positive by ELISA, and 12 (2.9%) were strongly positive by ELISA. 11 accepted MRI and 8 (72.3%) had NCC, including 5 with subarachnoid cysts, 5 with parenchymal vesicular cysts and 2 with parenchymal granulomas. Epilepsy and NCC are common among ring residents although it is unclear whether the risk is greater than in the general population as no controls were evaluated in this study. The high positive predictive value of ELISA in this population suggests a potential role for antigen screening of blood or urine to allow early detection and intervention of people with NCC, particularly those with subarachnoid cysts, the most malignant presentation.

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TAENIA SOLIUM AND NEUROCYSTICERCOSIS BURDEN AND DECREASED ACADEMIC PERFORMANCE ASSOCIATED WITH BRAIN INFECTION IN SCHOOL AGED CHILDREN, SOUTHWEST CHINA

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Neurocysticercosis (NCC) is caused by larval forms of the pig tapeworm, *Taenia solium*, invading the brain and has been shown to cause cognitive deficits in adults. We characterized *T. solium* exposure as well as NCC burden and resulting cognitive deficits in school-aged children living in

southwest China. We surveyed 3,038 students aged 10- 15 years in poor areas of southwest China, characterizing exposure history, collecting blood for *T. solium* serologic testing by ELISA, and administering a standardized math test. We characterized children as probable NCC cases if they had positive serologic testing and reported serious neurologic symptoms (seizures or recurrent headaches or weakness). We randomly selected a subset of students representing all possible combinations of serologic results and presence or absence of neurologic symptoms for in- depth testing that included brain MRIs and additional cognitive testing. 6% of children (175/2867) had positive serologies demonstrating exposure to *T. solium*, with some counties having prevalences higher than 15%. 15% (459/2953) of children reported consumption of undercooked pork in the month prior to the survey and 25% (761/3027) reported seeing encysted parasites in their meat in the past year. 14% (408/2781) reported being treated with a deworming medication within the past year. Children reported seizures (93 cases, 3%), severe headaches (>6 headaches per month, 241 cases, 8%), and recurrent extremity weakness (>6 episodes per month, 59 cases, 2%). 2% of children (65/2867) met criteria as probable NCC cases. Children classified as probable NCC cases had significantly lower math scores compared to their uninfected classmates (average score difference of 0.65 standard deviations, p=0.02). MRI scans on a subset of students revealed evidence of *T. solium* brain involvement in 9% (6/63). Our results suggest that *T. solium* infection and NCC are wide spread in school-aged children in southwest China. Children with NCC underperform academically and this may contribute to a cycle of poverty. Our results suggest the need for *T. solium* eradication efforts to decrease disease burden in school-aged children.

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EFFICACY AND ADVERSE EVENTS OF NICLOSAMIDE IN A LARGE SCALE CYSTICERCOSIS ELIMINATION DEMONSTRATION PROGRAM ON THE NORTH COAST OF PERU

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Taeniasis is an important parasitic infection as the immediate cause of neurocysticercosis (NCC). Mass drug administration with niclosamide (NSM) is one strategy to control the disease. NSM is reported to be 90-95% efficacious against taeniasis and is considered safe for use in endemic regions given that it is minimally absorbed and therefore poses no risk to individuals with NCC. However, there is little published information regarding treatment efficacy and adverse events of NSM when used in endemic community settings. We evaluated the efficacy and adverse events of NSM during a large-scale cysticercosis elimination demonstration program in Tumbes, Peru, in which we offered three rounds of mass treatment with oral NSM, at 4 month intervals, to 79,191 rural residents older than 2 years of age. We collected post-treatment stools after the first round of NSM to diagnose taeniasis using coproantigen ELISA, and collected an additional stool sample at 30 days for those with taeniasis to evaluate treatment efficacy. We visited all participants in their homes to collect information about adverse treatment events. At total of 158,201 doses were administered across all 3 rounds with 68,751 (86.8%) people receiving at least one dose. Of the 45,391 people who provided a post-treatment stool sample, 235 had taeniasis. 210 provided a follow-up stool sample and 59 had evidence of persistent infection after 30 days, representing a treatment efficacy of 71.90% (151/210). The prevalence of adverse events related to ingestion of NSM was 0.90% (1418/158,201), of which 98.73% (1400/1418) were of mild intensity. There were no severe adverse events. Abdominal pain was the most frequently reported adverse event (571/1418; 40.27%). Adverse events were more common among females [PR=2.33; 95% CI 2.06-2.65] and among those that received NSM in more than one treatment round [2 doses PR 1.18; 95% CI 0.97-1.44; 3 doses PR 1.46; 95% CI 1.27-1.67). NSM is a safe drug for use in mass drug administration although the treatment efficacy is substantially less than previously reported which may reduce control effect.

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VISUALIZING NEUROCYSTICERCOSIS AND THE IMPACT OF CYSTS ON EPILEPTOGENESIS USING INTERACTIVE 3D MODELS

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Neurocysticercosis (NCC) is a preventable, disabling disease that affects approximately 50 million people worldwide; it is in critical need of research, medical education and increased global awareness and has been classified as a neglected tropical disease by the World Health Organization. A person contracts NCC by ingesting eggs from *Taenia solium*, a parasitic tapeworm. The eggs migrate through the body tissues into the brain where they develop into cysts. Eventually, these cysts can trigger a massive inflammatory response in the host and debilitating symptoms emerge such as seizures and the development of epilepsy. Seizures are the most common symptom associated with NCC, occurring in 70-80% of infected individuals, and NCC is the most common cause of adult acquired epilepsy in the world. This research investigates whether 3D modeling and interactive visualization aid researchers in comparing the neural cysts of patients with NCC to better understand why some infected individuals develop chronic epilepsy while others suffer from isolated seizures or do not seize at all. Patient data was segmented out to create interactive 3D models, which were embedded in a user interface. Emphasis was placed on the location and stage of cysts and surrounding edema. Patient EEG data was represented in the 3D models to visualize which parts of the brain are involved in seizure activity. The final application was embedded in a globally accessible web based user interface. The user can rotate the models in 3 dimensions, control the transparency and visibility of the EEG data, and view patient lesions on a generalized brain to account for the high variability of neuroanatomy between individuals. This project helps researchers explore the relationship between cyst location, stage, and number and the development of seizures or chronic epilepsy. This project uses biomedical visualization to enable research on NCC and epilepsy through the creation of a multimodal interactive. It demonstrates the critical role of biocommunciation in shaping medical research, clinical decisions, and patient outcomes through visual translation of clinical data.

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EPIDEMIOLOGIC CHARACTERIZATION OF *HYMENOLEPIS NANA* INFECTION IN CHILDREN AGED 2 TO 15 YEARS OLD ON THE NORTH COAST OF PERU

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Hymenolepis nana is among the most common intestinal parasites and an important public health disease in impoverished areas given it's morbidity in children and ease of transmission. Understanding the risk factors for transmission in endemic regions may guide strategies to reduce the burden of infection. We conducted a cross-sectional secondary analysis of data collected during a large community-based study of 107 villages (73,161 people) in Tumbes, Peru, to evaluate risk factors for *H. nana* infection among children. Of the 20,249 eligible residents based on age (2-15 years old), 14,671 provided a single stool sample that was analyzed by sedimentation and microscopy for the presence of *H. nana* eggs. We used binomial family generalized linear models with log link to calculate crude and adjusted prevalence ratios of sociodemographic factors that may influence infection risk. The crude overall prevalence of *H. nana* infection was 7.61% (1124/14,761), and was slightly higher among males (8.59%; 95% CI 7.95-9.22) than among females (6.65%; 95% CI 6.08-7.21). In the multivariable model, the prevalence of *H. nana* infection increased 8% (adjusted prevalence ratio, aPR 1.08, 95% CI 1.06-1.09) with each additional year of age, was 30% higher among females than males (aPR 1.30, 95% CI 1.16-1.46), and 27% lower among rural dwellers compared to urban dwellers (aPR 0.73, 95% CI 0.64-0.84), after controlling for household clustering and other variables. Water source, particularly in-ground storage tanks filled by water truck, also increased the risk compared to piped water (PR 2.70, 95% CI 2.34-3.11), as did the lack of latrine (PR 2.00 95% CI 1.73-2.33). *H. nana* infection is a relatively common intestinal infection in northern Peru. Improvements to water and sanitation infrastructure may help reduce the burden of this and other intestinal infection in the region.

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EFFICACY OF SINGLE DOSES OF PRAZIQUANTEL 5-10 MG/KG FOR TAENIASIS UNDER CONTROLLED CONDITIONS IN RURAL COMMUNITIES OF THE NORTHERN COAST OF PERU

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Taenia solium has two stages, the adult tapeworm in the human intestine (Taeniasis) and the larval cyst in humans and pigs (cysticercosis). Praziquantel (PZQ) is considered the first line treatment for taeniasis, although there are concerns for potential adverse effects in people with neurocysticercosis (NCC) with viable cysts. Niclosamide is a safe alternative although its availability is limited. The objective of this study was to assess the efficacy of PZQ for intestinal taeniasis in an area endemic for *Taenia solium* at the usual single oral doses of 5 mg/kg or 10mg/kg (maximum dose 900 mg) under controlled conditions to ensure the safety of participants. Taeniasis was prospectively identified from the endemic region of Piura, Peru, in a series of mass stool screening interventions between 2011-2013. People with taeniasis 12-65 years old were eligible for participation and underwent a battery of screening tests to rule out NCC before treatment, including a) non-contrast brain CT scan to identify viable cysts, b) EITB for cysticercosis antibodies and c) cysticercosis antigen detection. Participants with viable cysts on CT or those with positive circulating antigen received niclosamide as an alternative to PZQ. The remaining cases received a single oral dose of PZQ at either 5 mg/kg or 10 mg/kg, with stool samples collected 30 days post treatment to evaluate cure. Of the 67 age-eligible cases, 10 refused treatment, 14 (21%) were given NSM because of positive antigen results. CT scan demonstrated 30 patients (44.7%) with parenchymal calcifications and apparently none with viable cysts. Of the remaining 43 participants with taeniasis, 33 (76.7%) received 5 mg/kg PZQ and 10 received 10 mg/kg of PZQ. Rates of cure as assessed in those who provided a 30-day follow-up stool sample were 26/29 (89.6%) for the 5 mg group and 10/10 (100%) for the 10 mg group. In conclusion, PZQ at 5 or 10 mg/kg was highly effective to treat *T. solium* taeniasis with few side effects in this selected subgroup of patients with no viable cysts on CT and negative circulating antigen.

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GEOSPATIAL ANALYSIS OF CYST BURDEN IN PIGS AS AN INDICATOR FOR LOCAL TRANSMISSION OF *TAENIA SOLIUM*

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Taenia solium is a parasite that is the leading cause of preventable epilepsy in the developing world. Taenia eggs are released into the environment through the stool of humans infected with an adult intestinal tapeworm (taeniasis), and cause cysticercosis when ingested by pigs or other humans. Ring strategies for the control of *T. solium* operate by screening and/or treating human for taeniasis if they live near pigs heavily infected with *T. solium* cysts. Traditionally, these heavily infected pigs have been diagnosed by examining the tongues of pigs for cysts, but higher sensitivity methods, such as improved serologic testing or ultrasonographic imaging, are needed in order to advance current control efforts. These tests require that we define thresholds of heavy infection that will perform best in the context of ring strategies. For this study, we performed necroscopic examination to determine the total body burden of viable *T. solium* cysts in pigs purchased from eight villages as part of a concurrent prospective ring intervention. Human stool was collected and processed for the presence of *Taenia* sp. coproantigen indicative of active taeniasis, and the geographic coordinates for each household were recorded. We assessed the prevalence of taeniasis inside rings of 50 and 100 meters around pigs of different cyst burdens in order to determine the rings that best indicated high-risk geographic foci for taeniasis. Of 152 seropositive pigs necropsied, 12 (8%) had ≥ 100 viable cysts, 9 (6%) had 10-99 cysts, and 27 (18%) had 1-9 cysts. There were 33 cases of taeniasis among all 1,323 people tested for a prevalence of 2.4%. Pigs with ≥100 cysts were significant indicators of local taeniasis when assessed at a distance of 50 meters (PR=2.74; 95% CI: 1.09, 6.91), but did not show an effect at greater distances. Pigs with fewer than 100 cysts did not significantly predict for local increases in the prevalence of taeniasis. The results of this study indicate that tests intended to diagnose heavily infected pigs should aim to detect pigs with at least 100 cysts. Lower detection limits are unlikely to yield additional cases of taeniasis in the context of ring interventions.

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PATHOGENESIS OF SEIZURES IN NEUROCYSTICERCOSIS: FROM CYSTICERCOTIC LESIONS TO SEIZURE SEMIOLOGY

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Neurocysticercosis (NCC) is the most common parasitic infection of the central nervous system and constitutes a major health problem. The aim of this study is to determine the concordance between semiologic manifestations and lesion topography in patients with NCC and seizures. In this retrospective cohort analysis patients with one or two NCC lesions in any stage were selected from participants in three randomized clinical trials of antiparasitic treatment. Out of 220 randomized patients, 58 (26.4%) had one or two lesions and 8 (13.8%) of them apparently had only primarily generalized tonic clonic seizures and were excluded, leaving 50 patients for analysis. Forty-two (84%) had the symptomatogenic zone, solely determined by seizure semiology, and at least one NCC lesion in the same sublobar brain region. Twelve (24%) patients had seizures semiologically arising from the peri-operculum region (perisylvian part of the frontal, parietal and temporal lobes), characterized by speech arrest, face motor or somatosensory seizures and epileptic vertigo or dizziness. Fifteen (30%) patients showed concordance between semiologic manifestations with the superior sublobar central region (parts of the frontal and parietal lobes), characterized by clonic motor or somatosensory seizures of extremities. In the 10 (20%) patients who had a calcified lesion and a viable cyst, the calcified lesions had not relation with the semiology of seizures. Out of 23 (46%) patients who had seizures after antiparasitic treatment was given in the randomized clinical trials, two (8.7%) had a new symptomatogenic zone and both showed a suitable semiological-topographical concordance for another viable lesion. In conclusion, roughly 85% patients with one or two NCC lesions and seizure history exhibit semiologic-topographic concordance suggesting a direct influence of the parasitic lesions in the very near surrounding brain tissue such the main sustainable cause of seizures. Follow-up studies including modern neuroimaging techniques and ictal-EEG to appropriate establishing the ictal onset zone should help us to better understand epileptogenesis in human NCC.

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A POTENTIAL CANDIDATE ENOLASE FROM *TAENIA SOLIUM* EXPRESSED IN BACULOVIRUS SYSTEM FOR INMUNODIAGNOSIS OF SWINE CYSTICERCOSIS

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Taenia solium, is a zoonotic parasite that infects both humans (as a final host) and swine (as an intermediate host). The pig acquires cysticercosis when it eats contaminated food with human feces with viable *T. solium* eggs. In endemic countries it is considered a serious public health problem. In the search for new antigens for immunodiagnostic of swine cysticercosis, we have annotated a glycolytic enzyme called enolase, whose function in Taenia species is not yet well understood. We have expressed the enolase of *T. solium* with their respective posttranslational modifications in the baculovirus-insect cell expression system (BES). We cloned the coding sequences in pFastBac HTA and the gene rearrangement was made in the baculovirus genome (bacmid) in *E. coli* DH10 Bac. We used bacmids for viral particles production and we obtained 3x108 viral particles in the infected sf9 insect cells. These were used to express 740 µg/mL of recombinant enolase (52 kDa), also it was identified by Western blot with polyclonal rabbit antibodies (1/5000) specific for enolase. The BES is efficient to express recombinant enolase with posttranslational modifications. Low titers of antibodies to recognize enolase suggest that they are highly antigenic. Finally, we evaluated the usefulness of the recombinant protein for the diagnosis of porcine cysticercosis through the detection of antibodies in sera samples of infected and non-infected pigs using ELISA test. We obtained 98,7 % of sensibility and 66,7 % of specificity.

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USEFULNESS OF TOURNIQUET TEST FOR DIAGNOSING DENGUE INFECTION IN ADULT

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Dengue infection becomes an important cause of fever among adult patients in the tropics. Tourniquet test (TT) is a classical bedside diagnostic tool which may aid clinician in the rural area where specific laboratory tests are not available. This study aims to determine the accuracy of TT in adult patients presenting with fever in the Bangkok Hospital for Tropical Diseases. A retrospective study was conducted in adult patients (age >18 years) presenting with acute febrile illness during 2012-2013. A TT performed using the standard technique once at presentation. Dengue infection was diagnosed by any of the following criteria: positive dengue specific NS-1 Ag. Total 1,157 adult patients with a median age of 30 years (18-96 years) were enrolled in this study. The male to female ratio was 1:1.4 and the mean duration of fever was 3.5 days. 259 cases (22.4%) had dengue infection. TT positive was found in 27.3% of patients. In our study, TT showed sensitivity 51.4%, specificity 79.5%, positive predictive value 41.9%, negative predictive value 85.0% and accuracy 73.2%. These parameters are varied by the day of fever on which TT was performed. The sensitivity of TT increased but the specificity decreased among patients with a longer history of fever. Patients with dengue infection were more likely to have positive TT than other febrile illness (OR 4.09, 95% CI 3.06-5.49). Among patients with dengue infection, the TT had no predictive value on the severity of dengue, risk of bleeding and length of stay in the hospital. Tourniquet test might be a useful predictor of the dengue infection in adult patient with acute febrile illness. However, among patients with confirmed dengue infection, tourniquet test had very little predictive value in the clinical course and outcome.

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SPIDR-WEB: AN NGS BIOTECHNOLOGY PLATFORM FOR DIAGNOSTIC, BIOSURVEILLANCE AND TRANSCRIPTOMIC APPLICATIONS

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We are transforming the field of infectious disease diagnostics with the development of the Sample Prep for Infectious Disease Recognition With EDGE Bioinformatics (SPIDR-WEB). SPIDR-WEB is a sample-to-result biotechnology platform that enables efficient use of next generation sequencing (NGS) for pathogen detection in clinical samples. NGS has become a powerful tool for detection and characterization of both known and emerging pathogens. The main advantage of NGS is its non-biased approach that identifies all organisms in a sample. This is in contrast to traditional molecular assays that force us to look for a set of specific pathogens. In most clinical samples, the relative abundance of pathogen nucleic acids (DNA or RNA) is vanishingly small. Therefore, vast amounts of sequence data must be generated and analyzed to identify rare pathogen sequences. SPIDR-WEB is a sample-to-result process that relies on efficient laboratory and in silico steps. Clinical samples mostly comprise non-informative host RNAs or abundant housekeeping gene transcripts. SPIDR-WEB incorporates removal of non-informative RNAs (RNR), thereby enriching all other RNAs, including those from pathogens. This step enables either higher sensitivity and specificity, or less expensive and faster sequencing. Our custom EDGE bioinformatics data analysis platform provides rapid read classification at all taxonomic levels, and reliably detects all organisms present in a sample. EDGE is an efficient process, as it uses databases with pre-computed signatures, instead of aligning sequencing reads to the entire Genbank. In addition to RNR and EDGE, SPIDR-WEB includes robust, inexpensive and rapid sample lysis, RNA extraction, and library preparation steps. We want to implement SPIDR-WEB in both research and clinical settings to support a multitude of applications, such as discovery of novel mechanisms and biomarkers, study host-pathogen interactions, improve vaccines and therapeutics, and complement current diagnostic tools and help improve their utility.

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EPIDEMIOLOGY OF LEPTOSPIROSIS AMONG PATIENTS PRESENTING WITH ACUTE FEBRILE ILLNESS TO LAKESIDE HEALTH CENTERS IN RURAL RWANDA

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Leptospirosis is an infectious disease caused by leptospires, which are transmitted directly or indirectly from animals to humans. Minimal data are available on leptospirosis epidemiology in Africa, including Rwanda. This study aimed to determine the frequency of leptospirosis infection as cause of acute febrile illness in patients presenting to three lakeside health centers in rural Rwanda. Included patients were 21 years old and above, with axillary temperature 37.50C or more, and a negative test for malaria. Serum samples were collected and tested with a Rapid Diagnostic Test {Standard Diagnostic Bioline) for anti-*Leptospira* IgG/IgM antibodies. Among 421 acute febrile patients, the study found a seroprevalence of 22.1% (93/421) for leptospira IgG positivity and an incidence of 5.5% (23/421) for IgM positivity; all IgM positive patients also had IgG antibodies and 85/93 cases with positive leptospira serology came from a single health center. Both IgG and IgM antibodies were strongly associated with lake swimming, livestock exposure and fish farming. Conjunctival suffusion was the physical examination finding most strongly linked with seropositivity. This study demonstrates the presence and localized endemicity of human leptospirosis infection in Rwanda. National mapping of prevalence, and enhanced clinician awareness supported by greater diagnostic resources, are needed to combat this under-recognized disease.

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PREVALENCE OF *TRYPANOSOMA CRUZI* AMONG NON-ISCHEMIC CARDIOMYOPATHY PATIENTS PRESENTING FOR CLINICAL MANAGEMENT AT THREE MEDICAL FACILITIES IN SOUTHEASTERN TEXAS, USA

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Infection with the parasite *Trypanosoma cruzi* (Chagas disease) causes a progressive dilating cardiomyopathy in approximately 30% of affected patients. Anecdotally referred to as the "silent killer", most patients are unaware of their disease status due to an asymptomatic or mild, non-specific acute infection period. Sudden cardiac disease is the first presenting symptom in 35% of patients with cardiac manifestations, and is the most common cause of mortality. Disease burden estimates suggest 7 million patients are living with chronic Chagas in Latin America. In the United States, we have a substantial burden of Chagas disease among immigrant populations, and we have recently started to recognize a growing number of locally acquired cases. Our current study aimed to understand the prevalence of *T. cruzi* infection in patients presenting for clinical management of known non-ischemic idiopathic cardiomyopathy

at one of three medical facilities. All housed in Houston, Texas, the three medical facilities serve distinct and unique populations: 1) Latin American immigrants living in Texas, 2) impoverished Texas residents with increased vector exposures, and 3) Texas residents with a high socioeconomic status. From this large cohort of cardiac patients, we identified a considerable proportion of previously undetected *T. cruzi* infection. We will discuss the clinical presentations and transmission risk factors of those patients who tested positive for Chagas disease compared to idiopathic cardiomyopathy patients with a negative serology. Our findings have important clinical implications for cardiologists practicing throughout the United States, as well as clinicians in other non-endemic countries providing healthcare to Latin American immigrants.

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OUTCOMES OF PATIENTS WITH SEVERE INFECTION IN UGANDA ACCORDING TO ADHERENCE TO WHO INTEGRATED MANAGEMENT OF ADOLESCENT AND ADULT ILLNESS FLUID RESUSCITATION GUIDELINES

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The World Health Organization's Integrated Management of Adolescent and Adult Illness (IMAI) recommendations for fluid resuscitation of patients with septic shock (IMAI-shock) and severe respiratory distress without shock (IMAI-SRDS) have not been validated. Therefore, we sought to describe outcomes in hospitalized patients in Uganda meeting these clinical criteria based on whether or not they received IMAI recommended fluid resuscitation. We performed a secondary analysis of data from a prospective cohort of severely septic adult patients admitted to Mbarara Regional Referral Hospital in Uganda that included the volume of intravenous fluids patients received during the first 6 hours of resuscitation. We selected patients meeting criteria for IMAI-shock or IMAI-SRDS and used logistic regression to determine predictors of outcomes. We evaluated 136 patients with IMAI-shock and 41 patients with IMAI-SRDS. During the first 6 hours of resuscitation, for patients with IMAI-shock, those that received IMAI recommended fluid volume (N=28) received more than those that did not (3L vs 1.5L, p<0.001) and there was no difference in mortality (30% vs 36%, p=0.788). Receipt of IMAI recommended fluid volume was associated with admission O2 saturation <90% (aOR 2.9, 95%CI 1.1-7.1, p=0.025) and in-hospital mortality was associated with wasting (aOR 3.9, 95%CI 1.4-11.0, p=0.026) and ambulation (aOR 0.2, 95%CI 0.09-0.6, p=0.005). For patients with IMAI-SRDS, those that received IMAI recommended fluid volume (N=9) received less than those that did not (0.5L vs 1L, p<0.001) and there was no difference in mortality (22% vs 57%, p=0.08). Receipt of IMAI recommended fluid volume and in-hospital mortality were both associated with a Glasgow Coma Scale score <15 (aOR 0.07, 95%CI 0.007-0.7, p=0.021; aOR 6.9, 1.04-45.7, p=0.045). In conclusion, IMAI recommended fluid resuscitation did not improve outcomes for patients with IMAI-shock or IMAI-SRDS. Further studies are needed to better understand the optimal resuscitation strategy for patients with severe infection in resource-limited settings such as Uganda.

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A RETROSPECTIVE STUDY OF MALARIA-RELATED DEATHS IN CHILDREN THAT DIED ON ADMISSION WITH SYMPTOMS OF FEVER MANIFESTATION IN A SECONDARY HEALTH CARE INSTITUTION IN WESTERN REGION OF GHANA

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Malaria contributes significantly to deaths in children. Children are vulnerable to severe consequences of malaria due to lack of anti-parasite and clinical immunity. Malaria causes death either directly being the underlying cause of death as in the case of cerebral malaria and severe malaria anemia (SMA) or indirectly by contributing to the cause as in the case of malaria in a child with pneumonia or hypoglycemia. This is a retrospective study which evaluated the malaria-related deaths among children who died on admission over a 3 year period. The study was done in the children's ward of Effia nkwanta hospital. A total of 223 dead children medical records were reviewed. Percentage mortality was 15.7% and Case fatality rate for malaria was 13.7%. All-cause mortality and malaria-specific deaths decreased from 21.5% and 24.3% in 2010 to 11.1% and 4.4% in 2012 respectively. 17.9% (40/223) tested positive for malaria with Cerebral malaria 37.5% (15/40), Severe malaria anemia 30 %(12/40), and Severe malaria 25% (10/40). A continuous unrelenting implementation of child survival and malaria control programs is very necessary to protect children from malaria and other childhood killer diseases.

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INFECTIOUS ETIOLOGIES OF FEBRILE ILLNESSES IN CAMEROON

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Fever is a common cause of patients seeking treatment in healthcare facilities in most tropical countries and poses a diagnostic and therapeutic challenge to healthcare workers in resource limited areas. Diagnosis of febrile illnesses in most malaria endemic countries mostly focuses on confirming or ruling out malaria. Thus, healthcare workers are often faced with the challenge on the course of action to take in treating febrile patients negative for malaria. The lack of information on the specific etiologic agents of non-malaria febrile illnesses prevents effective management of otherwise often treatable diseases. Despite their importance, there is no published data on the epidemiology of non-malaria febrile illnesses in Cameroon and their true burden remains unknown. In this study, we sought to identify pathogens that cause febrile illnesses in Cameroon. We recruited 551 febrile patients (6 months and older) in three different geographical regions of Cameroon. Blood and stool specimens were collected to perform rapid diagnostic test, ELISA, microsphere immunoassay, microscopy, culture and PCR to identify various etiologic agents of febrile illnesses. Of the 551 participants, 50% had malaria, 41.5% had one or more acute respiratory viral infections (influenza A (9%), influenza B (9%), adenovirus (10%), respiratory syncytia virus (17.5%), and parainfluenza virus (8%)), 7% had typhoid, 1.8% had acute toxoplasmosis, 18% had gastroenteric infections, 2% had dengue, 6% had West Nile virus infection, 0.5% had leptospirosis, 1% had acute

chikungunya virus infection, while 13% of the participants were co-infected with malaria and one or more non malarial pathogens. Moreover, 91% participants were presumed to have malaria based on fever, of which 41% were negative for malaria by PCR. Our results show evidence of non-malaria febrile illnesses in a malaria endemic region, which should be considered by clinicians in the differential diagnosis of febrile illnesses. But, lack of access to diagnostic tests impedes precise clinical management of febrle illnesses.

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EVALUATION OF SAFETY TOOL FOR AMBULATORY LEPROSY PATIENTS AT RISK OF ADVERSE OUTCOME

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Leprosy treatment requires a complex management approach consisting of ongoing laboratory monitoring, screening for factors that will adversely affect response to corticosteroids, engagement of allied health services, and prolonged follow-up. Given that leprosy is complicated to manage, a novel safety tool was developed and implemented in our practice in March 2015. Our objective was to evaluate its utility through retrospective chart review. We reviewed charts of patients with leprosy treated over a 3.5-year period: 3 years pre-implementation, and the 6-months following implementation. Outcomes included: loss to follow-up; monitoring of key laboratory parameters; allied health services engagement; baseline ophthalmologic assessment; and risk mitigation interventions such as prescription of GI and bone protection if on steroids. Seventeen patients with leprosy were treated during the enrolment period: 10 males (58.8%), and 7 females (41.2%). Of 17 patients enrolled, 8 were treated pre-implementation, and 9 post-implementation. Seven patients (41.2%) were classified as paucibacillary, and 10 (58.8%) as multibacillary. Five patients (29.4%) were lost to follow-up, all of whom were lost prior to implementation of the safety tool. One (12.5%) pre-implementation patient was sent for baseline ophthalmologic assessment vs. 8 (88.9%) post-implementation, (p=0.01). Only post-implementation patients received referrals for occupational therapy and social work. Seven (77.8%) patients were referred for in-home occupational therapy assessments and 33.3% (n=3) were referred for social work assessments. Laboratory parameters were routinely monitored for both groups. GI and bone protection were provided for all patients on steroids. Implementation of the safety tool has established a user-friendly method for systemizing all elements of care that are critical to appropriate management of leprosy. The tool has been particularly useful in ensuring the involvement of all consulting and allied health services necessary for optimizing outcomes of leprosy patients.

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EVALUATION OF A CLINIC-BASED QUALITY STRUCTURE FOR MEDICINES TO TREAT PARASITIC INFECTIONS

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Frequently used drugs in the Tropical Disease Unit (TDU) of Toronto General Hospital, including albendazole and ivermectin, are only available through the Special Access Programme (SAP) of Health Canada. As there are multiple points at which errors can occur in this process, a tracking system was implemented to ensure quality control of SAP-supplied medications and to evaluate the turnaround time (TAT) and success rate of SAP applications for drugs to treat common illnesses evaluated in the TDU. We aimed to evaluate the utility of this tracking system. A retrospective review of the TDU SAP logs for 2013-2015 was undertaken, and data including the TAT for each drug, incomplete notification rate (i.e., request by SAP for additional details before approval), and final approval rates over time were analyzed by one-way ANOVA or Yates' correction Chi square analysis, respectively. The mean TAT decreased progressively from 2013 to 2015, from 9.27 ± 10.3 days to 7.38 ± 8.03 days to 5.15 ± 1.93 days (p=0.04). The rates of incomplete notifications for albendzole in each of 2013 to 2015 were 25%, 54%, and 31%, respectively. For ivermectin, the incomplete notification rates in each of 2013 to 2015 were 4%, 16%, and 17%, respectively. Overall final approval rate for albendazole or ivermectin was 100% in all years. First time success and incomplete notifications differed between ivermectin and albendazole, with 87% of applications for ivermectin approved immediately compared to 64% for albendazole (p=0.0033). For the indication of Strongyloides hyperinfection (n=6), specifically, the mean TAT for ivermectin was 5.11 ± 0.694 days. It is critical that patients receive the necessary medications for their helminthiases , as rejections or delays in drug approval increase the likelihood of patient adverse outcomes including death. This is especially true for entities such as disseminated strongyloidiasis or ruptured hydatid cyst. Prior studies have demonstrated increased patient morbidity and mortality resulting from delays associated with the SAP drug approval process. Our clinic-based system may have contributed to the improved TAT noted over time.

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IMMUNIZATION AGAINST TETANUS DURING PREGNANCY: SEROLOGICAL INVESTIGATION FOR MATERNAL AND NEONATAL ANTIBODIES IN WEST REGION OF BURKINA FASO, BOBO DIOULASSO

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In Burkina Faso, neonatal tetanus often occurs often during aseptic childbirth and is related to infection of the umbilical cord in a baby born of an unimmunized mother. The Centers for Disease Control and Prevention (CDC) estimate that over 270,000 deaths occur annually worldwide as a result of neonatal tetanus. It accounts for about one-half of tetanus deaths in Burkina Faso. This study aims to assess risk factors linked to inadequate immunization against tetanus and immunity status of the newborn. One hundred venous blood plasmas were collected from pregnant women during eutocic childbirth and were compared with blood sample umbilical cordon from their newborns. The tetanus antibody titers were determined by enzyme-linked immunosorbent assay (ELISA kit; IBL International GMBH, Germany) in accordance with the manufacturer's recommendation. The results were reported in IU/mL, and they were standardized by comparing them to calibrated World Health Organization (WHO) reference sera. Overall, 100 women were assessed, 74% are illiterate, 57% are in age group 18-25 years old and 26% are on their second pregnancy. Most of these women, 93% have tetanus antibody titers upper than 1.00 IU/mL. Geometric mean of tetanus antibody titers was 3.66 (95%CI 3.13-4.28) in mothers and 3.87 (95%CI 3.28-4.58) in newborns. There is a significant correlation between antibody titer in the two groups (Spearman's coefficient=0.86, p<0.05). But there is no obvious link between tetanus antibody titers, the number of doses of tetanus vaccine given during pregnancy on the one hand (coeff=0.3, p<0.5) and the interval since the last dose on the other hand (coeff=0.3, p=0.85). Pregnant women and their newborns are well immunized against tetanus in Bobo Dioulasso. There is no reliable evidence related with gravidity, number of tetanus vaccine doses given during pregnancy and the tetanus antibody titer.

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EVALUATING TREATMENT OUTCOMES OF AMBULATORY LEPROSY PATIENTS RECEIVING OFLOXACIN-CONTAINING MULTIDRUG THERAPY REGIMENS

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There is an ongoing need to evaluate the efficacy of treatment options for leprosy, as the standard dapsone and clofazimine-containing multidrug therapy (MDT) continues to face barriers such as safety and tolerability. The fluoroquinolone ofloxacin has shown promise as an effective component of MDT, however, few data on the efficacy of ofloxacin-containing MDT for the treatment of leprosy in non-endemic areas are available. We evaluated the treatment outcomes of leprosy patients receiving ofloxacin-containing MDT regimens in our practice. We performed a retrospective chart review of all patients treated for leprosy in our practice over a 3.5-year period. Primary outcomes evaluated were cure rate; frequency of occurrence of leprosy reactions; frequency of dapsone-associated adverse effects; and treatment adherence. Analysis was descriptive, but data were also stratified by age, sex, spectrum of disease (paucibacillary vs. multibacillary), region of origin, and treatment regimen, and compared by odds ratios (ORs). During the enrolment period, 17 patients were treated with ofloxacin-containing MDT regimens: 10 males (58.8%) and 7 females (41.2%). Ten patients were classified as multibacillary, and 7 as paucibacillary. At the time of analysis, 5 patients (29.4%) had fully completed MDT, and all were deemed cured clinically, with a median duration of follow-up of 35 months (range 27 to 66 months). Five patients (29.4%) were lost to follow-up after completion of treatment, and 7 (41.2%) are still in active treatment. Ten patients (58.8%) experienced either ENL (n=3, 17.6%) or reversal reactions (n=5, 29.4%) or both (n=2, 11.8%). Of those receiving a dapsone-containing regimen (n=12), 5 (41.7%) developed clinically significant methemoglobinemia, and 2 (16.7%) developed hemolysis. Patients from the Philippines (n=5) were more likely to undergo reversal reactions than those from the Indian subcontinent or Africa (n=12) (p=0.028). Although limited by sample size, our results demonstrate high cure rates and positive treatment outcomes with ofloxacin-containing MDT, with reaction rates comparable to those documented in other studies.

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INTUSSUSCEPTION SURVEILLANCE AMONG CHILDREN BEFORE ROTAVIRUS VACCINE INTRODUCTION IN BAMAKO, MALI

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Intussusception (IS) is a major cause of bowel obstruction in infants that has been linked to infective agents and more recently to rotavirus vaccination. As rotavirus vaccine introduction proceeds in GAVI-eligible countries, where few studies of IS have been performed to-date, there is keen interest in documenting the epidemiology of IS. This retrospective study provides baseline data among children from 0 to 15 years with IS admitted to the major pediatric surgery unit in Bamako, Mali at the Hôpital Gabriel Touré (HGT) from 2009 to 2014 (when rotavirus vaccine was introduced). We reviewed operating room and hospital registers to identify cases who met the definition of IS at Level 1 of Diagnostic Certainty from the IS Brighton Collaboration Working Group (2004). During the 6 year study period, IS was diagnosed in 458 (6.1%) of 7,429 children hospitalized on the pediatric surgery unit of HGT; 66.8% were male and 61.6 % were younger than 1 year of age. The mean period incidence was 84.6 per 100,000 infants (range 29-81). The most common symptoms of IS were abdominal mass (78.4%), rectal bleeding (54.4%), vomiting (70.3%), crying/colic (54.8%) and abdominal distention (34.9%). IS was confirmed pre-operatively by sonogram in 381 (83.2%) and /or by X-ray in 7 (5.0%). Surgery was the sole therapeutic modality used, and was performed in 441 patients (96.3%), while 5 were lost to follow-up, 9 improved, and 3 died before surgery. Among 433 with surgically-confirmed IS, there were 23 spontaneous reductions, 309 manual reductions, 28 intestinal resections, and 35 ileostomies; 61 required a second surgical procedure. After surgery, 378 (82.5 %) of cases were discharged in good condition, 11 (2.4%) had complications and 64 (13.9%) died. Children who died were younger, had a longer duration of symptoms, and were more systemically ill. Intussusception is a relatively frequent occurrence in children under 1 year of age in Bamako, Mali and is associated with a high mortality. The ability to monitor changes in incidence related to vaccine is complicated by natural annual variation. Efforts are needed to improve outcome of IS and to detect possible vaccine-related cases.

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FIELD CHALLENGES IN CONDUCTING RESEARCH IN MULTIDRUG RESISTANT MALARIA AND PRE-ELIMINATION SETTINGS

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As efforts to eliminate malaria scale up, a range of practical challenges in conducting research in these settings arise which makes quality clinical research increasingly difficult. This analysis sought to gain a more comprehensive understanding of the difficulties encountered by research and field staff based in the field in these settings to improve the success of future research projects in malaria elimination settings. Reports from field-based studies were reviewed using content analysis to identify the challenges that were reported while conducting the research. In depth interviews were conducted with Village Malaria Workers (VMWs) involved in the research to identify challenges that may not have been reported in official reports. Informal interviews were conducted to provide more insight into the common themes identified from the report content analysis. As malaria elimination progresses, the smaller numbers of study participants available presents a range of practical field challenges that can impact upon the study outcomes. This is further affected by the selection of exclusion criteria. We found that in Western Cambodia migrant and mobile populations (MMPs) were frequently excluded from studies because of potential difficulties in study follow-up. This led to significantly reduced numbers for study enrolment and the exclusion of a critical high-risk group. We also also found that implementation challenges are reported sporadically and are often not formally reported. Conclusions This is the first study reporting the implementation challenges faced in conducting clinical trials in malaria elimination and drug resistance settings. The findings of the analysis will assist in informing the design of future projects to increase the likely success of these studies. The inclusion of MMPs as participants in future studies, although presenting some challenges will also reduce potential biases and makes the research more relevant for elimination in these settings.

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PARASITE AND *MYCOBACTERIUM TUBERCULOSIS* CO-INFECTION IN IMMIGRANTS

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Tuberculosis (TB) is the leading infectious cause of mortality worldwide, and parasitic infections are endemic in 21 of the 22 countries with the highest TB burden. Although data suggest that parasitic infections alter susceptibility to infection with *Mycobacterium tuberculosis* (Mtb), it remains unclear whether this association varies by parasite species. We aim to assess differences in susceptibility to Mtb infection and immune response to Mtb based on parasite species. Immigrants receiving routine clinical care at Boston Medical Center are enrolled (anticipated sample size of 400). Information on the social and medical history of participants is collected from medical records. Participants are tested for TB infection with the Quantiferon Gold-in-Tube (QGIT) assay and for parasites with stool microscopy and antigen testing and with serology for those with eosinophilia or relevant symptoms. QGIT supernatants are analyzed to quantify Th1, Th2, and Treg responses and compare these between individuals with protozoal infections, helminths, and no parasites. Thirty-nine participants have been enrolled to date: median age is 28 (range 10-76) and 25 (64.1%) are male. Of 36 with QGIT results, 8 (22.2%) are positive and, among 23 with stool testing results, 8 (34.8%) have at least one parasite. Among those with parasites, 7 (87.5%) have at least one protozoal infection (5 (71.4%) Blastocystis hominis, 1 (14.3%) Endolimax nana and Blastocystis hominis, 1 (14.3%) Endolimax nana, Dientamoeba fragilis and Blastocystis hominis), and 1 (12.5%) with schistosomiasis (based on serology). Comparing those with protozoa (n=7) to those with no parasites (n=15), 4 (57.1%) and 3 (20.0%) are QGIT positive (p=0.08). Those with protozoa are of similar age (median age 28 vs. 29) and more likely to be female (4 (57.1%) vs. 4 (26.7%), p=0.17) than those with no parasites. Immunologic analyses are pending. This ongoing study of newly arrived immigrant patients at Boston Medical Center will provide data to delineate the association between protozoa and helminth response to latent tuberculosis infection. Participant enrollment and data analyses are ongoing.

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Lauren A. Saag[1], Rachel L. Epstein[2], Jackson B. Mesick[1], Sondra S. Crosby[2], Sarah L. Kimball[2], Elizabeth D. Barnett[2], Natasha S. Hochberg[2]

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THE IMPACT OF SYSTEMATIC POINT-OF-CARE ULTRASOUND ON MANAGEMENT OF PATIENTS IN A RESOURCE-LIMITED SETTING

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Although target point-of-care (POC) ultrasonography has been shown to benefit patients in resource-limited settings, it is not clear whether a systematic POC ultrasound assessment in these settings can also lead to similar changes in patient management. A pre-defined systematic set of POC ultrasound scans were performed on inpatients at a tertiary referral hospital in Tanzania to see if this resulted in changes to patient management. Of the 55 patients scanned, an abnormality was detected in 75% (n=41), and a change in patient management was recommended or implemented on the basis of POC ultrasound findings in 53% (n=29). The main impact was earlier initiation of treatment due to more rapid and accurate diagnosis. Further research is warranted to determine whether systematic POC ultrasonography would result in improved patient outcomes in resource-limited settings.

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INTRODUCTION OF THE MISGAV-LADACH CAESAREAN SECTION TECHNIQUE TO A NIGERIAN TEACHING HOSPITAL

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When compared to the traditional Caesarean section technique, the Misgav-Ladach method for Caesarean section has been shown to be more efficient, leading to decreased operating time, peri-operative bleeding, and need for post-operative painkillers . We compared the two techniques sequentially after the introduction of the Misgav-Ladach method to a teaching hospital in Nigeria via a teaching DVD provided by the World Health Organization. 144 patients who underwent Caesarean-sections between March 2007 and November 2009 were compared. The first 72 received a traditional Caesarean section before the WHO training had been completed. The second group of 72 after the training received the Misgav-Ladach method. The two groups were analyzed with a multivariate analysis of covariance controlling for patient age and the acuity of the indication for surgery (emergent vs. urgent). Overall, mean surgical time was lower in the Misgav-Ladach group (55.8 minutes) compared to the traditional method group (72.3 minutes; $p < 0.001$). There were no significant differences in blood loss, number of transfusions, or pain medication doses

between the two groups. To examine differences in resident and attending use of the techniques; 25 resident cases were compared to 119 attending cases. The residents had longer surgical times (69.2 minutes) than attendings (58.9 minutes; p <0.034) there were no technique interactions. As such, the Misgav-Ladach group had significantly lower surgical times regardless of resident vs. attending physician. The Misgav-Ladach method appears to be faster than the traditional technique. In general, there were trends indicating less blood loss and pain medications in the Migav-Ladach group though only surgical time was significant. These differences could have systems implications , especially in low-to-middle income countries, where resources are less available. The fact that the training took place through a DVD and that the benefit was seen in both attendings and resident physicians shows promise for an effective means of disseminating the skills needed to incorporate this technique in areas of high need.

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NEW STRATEGIES FOR THE DEVELOPMENT OF ANTIVENOM THERAPIES TO TREAT SNAKEBITE

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Snakebite is a neglected tropical disease that affects ~1.8 million people each year. Of these cases, upwards of 94,000 deaths occur, primarily in the rural regions of south Asia and sub-Saharan Africa. The mainstay of snakebite therapy is antivenom - polyclonal antibodies from hyperimmune animal serum derived from horses or sheep immunised with small amounts of snake venom. Different antivenoms are produced to treat bites by different medically important snakes, as the venom toxins that cause pathology vary from species to species. This process of generating commercial antivenoms has remained largely unchanged for the past century and, consequently, these therapeutics have many limitations: (i) limited paraspecifity at treating bites by different snake species, (ii) low specificity in terms of the number of antibodies that are specific to venom proteins and (iii) high incidences of adverse reactions due high volumes of foreign protein being delivered to patients. Here we describe new experimental strategies that we have developed to produce 'next-generation' antivenoms that display increased paraspecific cross-reactivity and specificity to toxic snake venom proteins. Using 'omic' technologies to characterise toxin gene expression in the venom gland and toxin proteins in secreted venom, we have elucidated the variation in venom composition observed in many snakes of medical importance. This facilitates the identification of conserved regions of venom-encoding genes that can be used as antigens to stimulate the production of antibodies with paraspecific activities. We show that experimental antivenoms developed using few epitope-string DNA immunogens are capable of stimulating antibody repertoires with comparable cross-reactivity to traditional antivenom, and which are capable of neutralising venom-induced pathology *in vitro* and *in vivo*. Such approaches provide new tools amenable for the development of 'pathology-specific' or 'continent-specific' antivenoms that are based on mixtures of monoclonal antibodies and will be more specific and efficacious at lower doses than existing treatments for snakebite.

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MONITORING OF PATIENTS OPERATED FOR TRACHOMATOUS TRICHIASIS IN THE KAYES REGION OF MALI

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The Kayes region of Mali has historically had a large trachoma burden. Implementation of the World Health Organization's SAFE strategy (Surgery, Antibiotics, Facial cleanliness and Environmental improvements) has led to significant reductions in trachoma prevalence. The Mali national program has a long standing surgery program to treat the trachomatous trichiasis (TT) cases and reduce the estimated national TT backlog. High quality data regarding surgical outcomes are essential to timely and accurate evaluation of trachoma programs. The aim of this study was to assess the quality of TT surgery by following up patients who underwent TT surgery in Kayes region, Mali, three to six months following the operation. A cross-sectional study was conducted in 8 health districts in Kayes region from June 2014 to February 2016. To be eligible patients must have been operated by mobile surgery teams within three to six months prior to the field trip and on the national surgery record. Fifteen to 20 patients were randomly selected from the national program's operated patient registry for each field visit. In total, 206 patients were enrolled and completed a structured interview and ophthalmological assessment. Concordance of collected data with that sent to the PNSO was verified for each patient interviewed. 143 of the 206 enrolled patients (69.4%) were interviewed, with a median age of 62.5 (range 9-94) and a ratio of 2.6:1 for females to males. Among the 143 patients interviewed, 27 (18.9%) patients have not been operated. Of the 116 patients operated for TT, in 24 (20.7%) cases, the reported operated eyelid did not match the actual operated eyelid. Suture removal did not occur in the recommended timeframe in 16 cases. Post-operative TT was found in 33/116 (28.4%) patients, and 28/116 (24.1%) had continued tearing in the operated eye after surgery. This study underscores the importance of post-operative follow-up to verify data completeness and quality, as well as surgical outcomes of patients. Refresher training for operators and TT surgery supervision must be strengthened to ensure high quality TT surgery to achieve trachoma elimination in Mali.

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PARASITIC DISEASES IN CAMBODIA: A NATIONAL SEROSURVEY OF WOMEN 15 TO 39 YEARS OF AGE BY MULTIPLEX BEAD ASSAY

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A national survey of Cambodian women of childbearing age was conducted in 2012 to assess immunity to vaccine preventable diseases such as polio, measles, and rubella. The availability of a nationally representative serum panel offered an opportunity to measure IgG

antibody levels to a number of parasitic diseases using a multiplex bead assay previously developed in our lab. Recombinant antigens from *Plasmodium falciparum*, *P. vivax*, *Toxoplasma gondii*, *Taenia solium*, *Strongyloides stercoralis*, and lymphatic filariasis were included in the multiplex assay panel, and national and regional prevalence values were estimated from the IgG antibody data. Few women were positive for cysticercosis or toxoplasmosis (<6% seroprevalence) and no geographic clustering was obvious. Malaria and lymphatic filariasis were mainly confined to the North region of the country with several distinct hotspots. Infection with S. stercoralis was widespread in the population (45.9% prevalence): urban residents (32% seroprevalence) had lower levels of infection than rural residents (50% seroprevalence). Integration of multiplex bead assays into nationally representative population serosurveys can provide valuable information on the presence, prevalence, and distribution of parasitic disease and can be used to evaluate the impact of public health interventions.

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A PROSPECTIVE STUDY OF SCABIES OUTBREAKS IN TEN RESIDENTIAL CARE FACILITIES FOR THE ELDERLY

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Scabies is a global health problem and common in closed communities. It represents a significant problem in residential care facilities (RCF) for the elderly in the UK and other countries. Clinical presentations in the elderly can differ from those in younger individuals. In RCFs where a scabies outbreak was suspected and prior to treatment, two physicians including a dermatologist performed examinations of residents, including dermoscopy and skin scrapings. . Scabies diagnosis was classified as definite/probable/possible. Outbreaks were defined as ≥2 suspected cases. Residents were treated with a topical scabicide twice. A second visit after treatment was performed. 230 residents were examined across 10 RCFs between February 2014 and February 2015. The median age was 86.9 years and 175 (76.1%) were female. 157 individuals (68.3%) had dementia. 61 (26.5%) were diagnosed with scabies. Of these 3 had crusted scabies. The median number of cases/RCF was 6 (2-11). The number of cases of scabies categorised as definite was 8 (13.1%). The mite was visualised using dermoscopy in 7 cases (11.5%) and identified on skin scraping in 3. Burrows were detected in 41.0% of cases. 31 (50.8%) individuals were asymptomatic. Dementia was significantly associated with having scabies (OR=2.37 95% CI 1.37-4.09). At the second visit (median interval of 44 days, range 37-81 days) there were no new cases of scabies. 10 individuals diagnosed with scabies at the first visit were identified as still having possible (8) or probable (2) scabies. Scabies is a difficult diagnosis to make in this population with more than half of affected individuals being asymptomatic often with subtle clinical signs. Dermoscopy and skin scrapings were of limited value in identifying affected individuals. This is the first study to confirm that dementia is a risk factor for scabies in this group. Our findings are of potential relevance to the understanding and management of scabies in highly endemic communities, RCFs in tropical settings and other closed communities. Elderly members of communities should be carefully examined as they may have no symptoms or typical signs.

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BACTERIAL ETIOLOGY AND OUTCOME OF CHILDHOOD LIFE THREATENING INFECTIONS IN THE GAMBIA: EUCLIDS IN WEST AFRICA

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Despite the importance of severe bacterial infections as a major cause of morbidity, disability and mortality in African children, data on the factors contributing to poor outcome of severe childhood bacterial infection in West African children is limited. We aimed to prospectively identify factors related to poor outcome. Patients aged 1 month to 16 years presenting with suspected sepsis and/or severe focal infections to two urban hospitals in The Gambia recruited for the European Union Life-threatening Infectious Disease study (www.euclids-project.eu) were part of this analysis. Basic demographic data, clinical features, outcome and pathogen identified using standard bacterial culture and molecular diagnostics were documented. From January 2013 to September 2015 we recruited 411 children, 96(23.3%) developed poor outcome; 54(13.1%) died and 42(10.2%) developed sequelae. The common bacterial pathogens identified were *Staphlococcus aureus* (37,24.7%), *S. pneumoniae* (31,20.7 %), *Neisseria meningitidis* (25,16.7%), and *Haemophilis influenzae* (20,13.3 %). The factors that were associated with poor outcomes were young age, presence of a cor-moridity, prior stay at another health facility and duration of symptoms prior to presentation. More than half of the mortality cases were less than 24 months with median age (IQR) of 16.3(5.9-60.0) months and sepsis was present in 39/54(72.2%) of those who died (p=0.004). 29/54 (69%) died within 48 hours of admission and gram negative organisms were the commonest(12/42(22.2%) associated with mortality. Abnormalities in the musculoskeletal system and central nervous system at presentation were more often associated with development of sequelae. Poor outcome is common (23.3%) in childhood life-threatening infections. A potential vaccine preventable disease was found in 27/96 (28.1%) of those with poor outcome. The attainment of MDG 4 of reduction of childhood mortality requires a review and improvement of vaccine policies as well as research into the production of novel effective vaccine against S. aureus.

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MALARIA PREVENTIVE MEASURES DURING ROUTINE CARE AMONG CHILDREN WITH SICKLE CELL DISEASE IN MALAWI

Graham Ellis[1], Godwin Chipoka[2], Pilirani Mafunga[2], Christopher Stanley[2], Tisungane Mvalo[2], Portia Kamthunzi[2], Isobel Kambalami[2], Peter Wasswa[3], Kate Westmoreland[4], Seyed Nouraie[5], Nigel Key[4], Kenneth Ataga[4], Satish Gopal[2]

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The contribution of malaria to sickle cell disease (SCD) morbidity in sub-Saharan Africa is unclear, particularly for children receiving chronic care including malaria preventive measures. In a Malawian pediatric SCD cohort, we describe use of preventive measures and prevalence for malaria, and explore associations with levels of hemoglobin (Hb) and lactate dehydrogenase (LDH). Weekly sulfadoxine/pyrimethamine (SP) was the local standard-of-care for malaria chemoprophylaxis. SP adherence, bed net use, and malaria history were assessed using a standardized survey. Overall, 94 children with SCD were evaluated with mean age 8.2 (SD 4.5) and median Hb 7.2 g/dL (IQR 6.6-7.7). 92 (98%) were receiving SP at evaluation. Of these, 53 (58%) reported perfect adherence to SP and 88 (96%) reported adherence to ≥75% of doses. The most common reasons for missed doses were running out of SP and guardian traveling. 66 (70%) reported regular bed net usage, with costs reported as the

major barrier to nonuse. 6 (6%) reported a history of malaria, and of 72 cross-sectional blood smears collected over three months including the transmission season, no malaria parasites were seen. Neither self-reported history of malaria, Hb, nor LDH correlated with SP adherence or bed net use. In conclusion, we observed high rates of adherence to SP and bed net use in a pediatric SCD cohort in Lilongwe. Self-reported malaria was very infrequent and laboratory-confirmed malaria was not detected, suggesting good effectiveness of malaria preventive measures in this population under routine care conditions.

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PRE-TRAVEL HEALTH CARE AMONG PEDIATRIC U.S. MILITARY BENEFICIARIES

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An increasing number of children are traveling to developing countries, placing them at risk for travel-related infections. There is limited data comparing pre-travel care, exposures and illnesses in pediatric and adult travelers. We used a prospective, observational cohort of US Department of Defense beneficiaries traveling overseas (TravMil), to compare pediatric and adult travelers using a case-control methodology. Pediatric subjects were matched 1:1 with adult military dependents by travel region, malaria risk at destination, and duration of travel. Outcomes of interest included pre-travel preventive care and associated compliance, travel exposures, and reported illnesses. 83 pediatric and adult subjects were matched. The median ages of pediatric and adult subjects were 11 years (IQR: 7-16) and 63 years (IQR: 44-67) years, respectively. Common regions of travel included South-East Asia (42%), South/Central America (34%), and Africa (26%). Pediatric travelers was more likely than adults to visit friends and relatives (39% vs. 10%; $p < 0.05$). Travel related vaccine coverage rates were similar, except Hepatitis A (pediatrics: 90% vs. adults: 71%; $p < 0.05$) and B (84% vs. 48%; $p<0.05$). Malarone was the most common antimalarial prescribed in children and adults, with no difference in non-compliance. Poor compliance with skin insect repellent use (55% vs. 67%), and frequent skin exposures including insect bites (94% vs. 89%), animals bites or scratches (52% vs. 27%) were reported among pediatric subjects ($p<0.05$). Subjects < 10 years of age were less likely to be prescribed antibiotics (RR=0.65; 95% CI: 0.50-0.86) and anti-diarrheals (RR=0.09; 95% CI: 0.03-0.28) for TD self treatment than adults, despite similar rates of high risk behavior for TD acquisition. Strategies to improve compliance with preventive measures, and standardization of TD treatment guidelines are needed for pediatric travelers.

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IRON DEFICIENCY IS COMMON IN UGANDAN CHILDREN WITH SICKLE CELL ANEMIA

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African children have a high prevalence of sickle cell anemia (SCA), which causes significant morbidity and early mortality. Previous studies of iron deficiency (ID) and the degree to which it contributes to anemia in children with SCA in malaria endemic areas have produced conflicting results. Our objective was to determine the prevalence of ID and its relationship to erythrocyte parameters in Ugandan children with SCA living in a malaria endemic area. 205 Ugandan children <4 years of age with SCA were assessed for ID by testing plasma levels of four iron-related biomarkers (ferritin, soluble transferrin receptor (sTfR), C-reactive protein (CRP), and hepcidin) by ELISA. ID was defined using previously established cutoffs: (1) ferritin <12 µg/L if CRP <10 mg/L or ferritin <30 µg/L if CRP ≥10 mg/L; or (2) hepcidin <5.5 ng/mL. Iron markers collectively reflected low iron stores and poor erythrocyte iron availability. Median (IQR) values for ferritin, sTfR, hepcidin, and CRP were 12.2 µg/L (7.5-26.2); 7.2 mg/L (5.5-9.0); 7.8 ng/mL (1.2-23.2); and 6.7 mg/L (2.6-16.7), respectively. The prevalence of ID was high (62.9% by ferritin cutoff, 42.4% by hepcidin cutoff). Children with ID as defined by low ferritin had similar hemoglobin levels (mean (SD)) to children without ID (7.6 g/dL (1.0) vs 7.4 g/dL (1.0), $p=0.12$) but had a lower MCV (78 fL (10) vs 82 fL (8), $p=0.005$). The data provide strong evidence that ID is common in children with SCA in a malaria endemic area. Studies are needed to assess the clinical and neurodevelopmental consequences of ID in children with SCA in malaria endemic areas, and to assess whether iron supplementation can be given to children with SCA in malaria endemic areas without increasing their risk of malaria and other infections.

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ENTERIC PATHOGENS AND FECAL BIOMARKERS OF GUT INFLAMMATION IN ASYMPTOMATIC INFANTS AND IMMUNE RESPONSE TO ORAL POLIOVIRUS VACCINE

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Oral poliovirus vaccine(OPV) immunogenicity is sub-optimal, in developing countries with high enteric infection prevalence. Enteropathogen presence in asymptomatic children in such settings is hypothesized to lead to impaired oral vaccine efficacy. We studied the relationship of enteropathogens and biomarkers of gut inflammation in asymptomatic 6-11 month old Indian infants with the immune response to a dose of monovalent OPV3 (mOPV3). Infants included were from a randomized clinical trial evaluating effect of a short course of azithromycin on mOPV3 seroresponse (CTRI/2014/05/004588). Enteropathogen profile in these infants (n=729) was evaluated using Taqman array card assays for enteropathogens, including bacterial, virus and parasite targets, from stool samples collected on the day of mOPV3 vaccination. In a subset of infants (n=299), fecal biomarkers of gut inflammation like calprotectin, myeloperoxidase, alpha-1antitrypsin and neopterin were estimated. Immune response to a mOPV3 dose was assessed by serum neutralization assays for anti-poliovirus 3 antibodies on samples collected 21 days after vaccination. Significantly higher proportion of infants failing to seroconvert were found to be infected with one or more enteropathogens on the day of the vaccination (94.3% of PV3 seronegatives vs 86.8% of seropositives, OR=2.5, 95CI=1.43-4.55). Enteric viruses were found in a significantly higher proportion of seronegative infants compared to seropositives (62% vs 46%, OR=1.9, 95CI=1.4-2.59). The difference with respect to bacterial enteropathogens or intestinal parasites even at higher pathogen loads was not significant. Among enteric viruses, non-polio enteroviruses (NPEV) were significantly associated with non-response to mOPV3(44% in seronegatives vs 29% in seropositives, OR=1.9, 95CI=1.4-2.6). Levels of fecal biomarkers like calprotectin, MPO and alpha-1AT were higher among seronegatives compared to seropositives, however, this difference was not significant. To conclude, this study adds to the evidence of association of enteric viruses, specifically NPEV, with OPV non response in developing settings.

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DEMOLISHING ACCESS BARRIERS TO HEALTHCARE IN TWO DIFFERENT CHAGAS DISEASE SCENARIOS: ENDEMIC AND NON-ENDEMIC AREAS IN ARGENTINA

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Approximately 1.6 million people are infected with *Trypanosoma cruzi* in Argentina and less than 1% has access to etiological treatment. Within the country there are three distinct epidemiological scenarios that can be characterized for Chagas Disease (CD): 1) areas with a recent history of vector transmission (VT) currently controlled with entomological surveillance (S1); 2) areas with uncontrolled VT and/or without current entomological surveillance (S2), and 3) a historically non-endemic area (S3). In both in SI and in S3, it is necessary to conduct actions of timely diagnosis and treatment (D&T) of affected individuals; which is where this study was conducted. It is worth mentioning that in S2 these actions should not be promoted given the risk of possible re-infection through vector transmission. According to the recommendations of the Pan American Health Organization, a model of D&T was developed for the first level of healthcare. In order to achieve this, it was first necessary to provide primary healthcare centers with the technical capacity to be able to treat the patient in one place, minimizing the economic burden on the families and loss to follow-up. Mundo Sano participated in the training and motivation of the healthcare teams and provided the equipment to allow the centers to perform electrocardiograms in site. The process of D&T was free of charge (including clinical analysis, medical consultations and anti-parasitic drug). As of March 2016 a total of 12,096 people were diagnosed, of which 1,101 were positive for CD and 999 of these were treated. This experience shows the great potential of the first level of healthcare to provide access to D&T for CD.

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A NOVEL ELECTRONIC ALGORITHM USING HOST BIOMARKER POINT-OF-CARE TESTS FOR MANAGEMENT OF FEVER IN UNDER-FIVES IN RESOURCE-POOR SETTINGS (E-POCT): A CONTROLLED, NON-INFERIORITY STUDY

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Infections cause the majority of deaths in under-fives in resource poor settings. Management of febrile illnesses at outpatient level remains inadequate and antibiotic overuse is a great challenge. The objectives were to determine whether a novel, electronic algorithm using point-of-care test results (e-POCT) is noninferior to a validated electronic algorithm derived from IMCI (ALMANACH) in treating fever in under-fives and to compare the proportion of antibiotic prescription between the two algorithms. E-POCT is an electronic algorithm developed by our group based on current evidence of pediatric fever management. It is built into an android application, which guides through the entire consultation and recommends treatment based on a few clinical signs, as well as point-of-care laboratory tests. We performed a randomized, controlled non-inferiority study of patients aged 2-59 months presenting with fever (axillary temperature ≥37.5°C) at 10 outpatient clinics in Dar es Salaam, Tanzania. The primary outcome was a noninferiority comparison between e-POCT and ALMANACH on the proportion of clinical failure by day (D) 7 of follow-up. Noninferiority would be declared if the percent of clinical failure with e-POCT was no worse than the proportion of clinical failure with ALMANACH, within statistical variability, by a margin of 3% in a modified intention to treat analysis. The secondary outcome was the comparison between both arms on the proportion of antibiotic prescribed on D0. We enrolled 1583 patients into the e-POCT-, and 1581 into the ALMANACH-arm between December 2014 and February 2016 with lost to follow-up of 0.5% and 0.8%, respectively. The percent of clinical failure by D7 was 2.1 for e-POCT and 3.4 for ALMANACH. The 97.5% lower confidence limit was a -2.4 difference in percent, establishing noninferiority. The Antibiotics were prescribed at D0 in 10.9% of patients (95% confidence interval [CI] 9.4-12.5) using e-POCT, compared to 29.0% using ALMANACH (CI 26.8 - 31.3). In conclusion, e-POCT is noninferior to ALMANACH in terms of clinical outcome by D7 while significantly reducing (by 62%) the proportion of antibiotic prescription at D0.

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ACTIVITY OF CRUDE EXTRACTS AND CHROMATOGRAPHIC FRACTIONS OF *DANIELLIA OLIVERI* AND *PSOROSPERMUM FEBRIFUGUM* AGAINST ADULT *BRUGIA PAHANGI*

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Lymphatic filariasis (LF) caused by *Brugia malayi*, *B. timori*, and *Wuchereria bancrofti,* potentially affects an estimated 1.2 billion people, with over 120 million people infected, and about 40 million people disfigured and incapacitated by the disease. The disease manifestations include painful and profound disfigurement of the skin, lymphedema, elephantiasis and scrotal swelling which may eventually lead to permanent disability. Hence, individuals infected not only suffer physical disability, but also suffer mental, social and economic losses, thus resulting in depression, stigma and poverty. Current control of LF relies on mass drug administration either a combination of ivermectin and albendazole or DEC and albendazole. However, these drugs are only effective against the microfilariae (mfs), with limited activity against the adult worms, which may live for up to 18 years. Effective control is therefore hindered by the lack of adulticides (macrofilaricides). There is a pressing need for new macrofilaricidal compounds. Medicinal plants used in traditional medicine have been identified to play a potential role in the remedy of diseases. Preliminary studies in our lab showed crude extracts of *Daniellia oliveri* and *Psorospermum febrifugum* inhibited adult *B. malayi* motility, when the Worminator system was used. In this study, we screened crude extracts and chromatographic fractions of D. oliveri and I for activity against adult I, a suitable animal model for *B. malayi*. Remarkably, the active crude extracts had IC50 values in the range of 44 - 154 µg/mL, the cleaned-up extracts had IC50 values in the range of 27 - 331 µg/mL, and the active chromatographic fractions caused a 100% inhibition of motility when tested at 300 µg/mL. This indicates that, *D. oliveri* and *P. febrifugum* may serve as sources of lead compounds for the development of the much needed macrofilaricidal drugs.

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FACTORS IMPACTING THE DETECTION OF *BRUGIA MALAYI* DNA WITHIN THE EXCRETA/FECES OF EXPOSED MOSQUITOES

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Recent experiments have indicated that the DNA of both *Brugia malayi* and *Plasmodium vivax* parasites is detectable in the pooled excreta/feces of vector mosquitoes. Utilizing real-time PCR, proof-of-concept work has demonstrated the high-throughput potential of this alternative approach to molecular xenomonitoring (MX). Yet despite these encouraging results, excreta/feces testing remains largely unexplored, and further work is required to more completely evaluate this novel methodology and gage its operational feasibility. Accordingly, we have performed comparative studies, aimed at assessing the detectability of parasite DNA within the excreta/feces obtained from mosquitoes under a variety of experimental conditions. Analyses have evaluated the impact of mf-blood density on parasite detection, while also examining DNA detection rates at various time-points post-exposure. The potential for DNA detection within the voided material of recently blood-fed, gravid, and host-seeking mosquitoes has also been explored, helping to better inform future trapping efforts intended for excreta/feces collection. Furthermore, as non-vector species clear *B. malayi* rather than harboring its development, the detectability of parasite DNA within the excreta/feces of non-vector mosquitoes was evaluated and results were compared with those for vector hosts. While a number of important questions remain to be addressed, the results described here provide a foundation for future studies aimed at operationalizing this novel approach to MX.

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PLACENTAL EXPRESSION OF IRON TRAFFICKING GENES IN THE CONTEXT OF HUMAN HELMINTHIASIS

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Iron deficiency during pregnancy and early infancy is a leading cause of poor growth and neurocognitive delays in childhood. Pregnant women and children in low and middle income countries (LMICs) are at increased

risk for iron deficiency due to iron poor diets and extra corporeal iron loss in stool due to helminthiasis. Herein, we examined the expression of ferroportin (FPN) and ferritin, key genes involved in iron trafficking across the placenta. Ferritin is the main storage protein for intracellular iron and FPN has been implicated at the primary exporter for iron from the placenta into fetal circulation. We isolated RNA from whole placental tissue stored in RNAlater (n=72) collected as part of a randomized controlled trial in Leyte, the Philippines, evaluating the impact of Praziquantel treatment during pregnancy on birth outcomes. Total RNA was reverse transcribed using random primers, and FPN, ferritin and 18S gene expression assessed using quantitative real-time PCR. FPN and ferritin gene expression were highly correlated in this cohort. Neither FPN nor ferritin gene expression in the term placenta was altered by Praziquantel treatment or maternal anemia status. Women who were infected with T. trichuria displayed significantly lower levels of both ferritin and FPN gene expression in the placenta. In addition, maternal levels of hepcidin, a key regulator of iron metabolism, at 32 weeks gestation were negatively associated with FPN gene expression in the placenta, while cord blood hepcidin was not associated with either FPN or ferritin gene expression. FPN expression was also positively associated with gestational age, and both FPN and ferritin expression were positively associated with birth weight in this sample of infants. Taken together, these data suggest that maternal factors such as trichuria infection and high hepcidin levels may have a greater influence on transplacental iron flux than fetal factors. In addition, effective iron trafficking is instrumental to successful pregnancy, and placentas challenged to transport adequate iron may result in newborns with lower birthweight.

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EFFICACY OF SINGLE AND REPEATED ORAL AND SUBCUTANEOUS DOSES OF FLUBENDAZOLE IN *LITOMOSOIDES SIGMODONTIS* INFECTED JIRDS

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Flubendazole (FBZ) is a promising macrofilaricidal drug candidate for the elimination of onchocerciasis. Recently a new bioavailable oral formulation was developed and its efficacy was investigated in the jird *Litomosoides sigmodontis* model. Microfilariae-positive jirds were divided into 11 groups of 12 jirds each and FBZ was tested at single (40 mg/kg) or repeated (2, 6 or 15 mg/kg for 5 or 10 days) oral (OR) doses and at single subcutaneous (SC) injections (2 or 10 mg/kg). Positive controls received 5 SC injections at 10 mg/kg, negative controls remained untreated. Jirds were euthanized 8 weeks post treatment end for adult worm counts. Single and 5 x 10 mg/kg SC FBZ completely cleared the adult worms in all animals (100%). Single 2 mg/kg SC and 10 x 15 mg/kg OR FBZ reduced the adult worm burden by 94% and 90%, respectively; single 40 mg/kg OR and 5 x 15 mg/kg OR by 80 and 85%, respectively. At necropsy, all animals in the SC groups had no detectable microfilariae within the peripheral blood, while the oral FBZ treatment regimens reduced the microfilaremia in a dose and duration dependent manner. FBZ was measured in plasma by LC/MS/MS. After OR, AUC 0-24h increased dose proportionally from 2 to 40 mg/kg and was similar between days 5 and 10. After SC, FBZ was slowly released from the injection site and plasma levels remained constant up to necropsy. Results of this study demonstrate that single and repeated SC injections and repeated oral administrations of FBZ have an excellent macrofilaricidal effect and achieve efficacies of ≥ 90% adult worm reduction. Histopathological analyses of the remaining female adult worms could be considered to determine whether a permanent sterilization was achieved, which would guarantee that the transmission is stopped and microfilariae-driven pathology in Onchocerciasis is reduced.

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LACK OF SIGNIFICANT MICROFILARICIDAL EFFICACY OF PARENTERAL OR ORAL BIOAVAILABLE FLUBENDAZOLE FORMULATIONS IN A *BRUGIA MALAYI* MICROFILARAEMIC MOUSE MODEL

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Flubendazole (FBZ) is a validated macrofilaricide in preclinical models of lymphatic filariasis (LF) and onchocerciasis when administered by multiple subcutaneous injections. A new amorphous solid dispersion (ASD) formulation of FBZ, with improved oral bioavailability, has been developed by Janssen. This formulation is currently undergoing testing in rodent models of filariasis to evaluate its *in vivo* efficacy as a candidate macrofilaricide. Preclinical macrofilaricides also require evaluation of direct microfilaricidal activity, especially considering potential indications in loiasis co-endemic foci, where severe adverse events have been reported following ivermectin (IVM) treatment. The *Brugia malayi* microfilaraemic mouse model is a validated screen to test activity of filaricidal compounds in inducing rapid decline of circulating blood mf *in vivo*. The purpose of this study was to assess whether single dose oral FBZ ASD (2 and 40 mg/kg) has direct microfilaricidal efficacy in SCID mice intravenously infused with *B. malayi* mf. In parallel, efficacy of standard FBZ suspension given by injection was evaluated (10mg/kg x 5 days). Human bioequivalent oral IVM (0.2 mg/kg) induced 81% average reduction in circulating mf at +48h compared with pre-treatment levels (n=5, P=0.0003). Circulating mf did not significantly decline at +48h in either single oral dose regimens (12 or 49% reductions in 2 or 40 mg/kg dose groups) or multiple injections of FBZ (35% reduction). Reductions observed in FBZ groups were similar to reductions in vehicle control circulating mf at +48h (38%). After 7 days, microfilaraemias were reduced significantly in single dose IVM group only compared to vehicle controls (85% efficacy, n=5 / group, F=3.733, P=0.0234). FBZ groups showed 0% efficacy when compared with vehicle controls. In conclusion, this counter-screening model demonstrates a lack of direct microfilaricidal activity of parenteral or oral FBZ formulations against circulating *B. malayi*. It is therefore unlikely that such bioequivalent FBZ regimens would mediate substantial direct effects against bloodborne microfilarial infections of humans.

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INDUSTRIAL SCALE SCREENING OF 1.3 MILLION COMPOUNDS IDENTIFIED 14 NOVEL CHEMOTYPES AS PROMISING NEW LEADS FOR THE TREATMENT OF LYMPHATIC FILARIASIS AND ONCHOCERCIASIS: A COLLABORATION BETWEEN THE ANTI-WOLBACHIA CONSORTIUM AND ASTRAZENECA

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A screening collaboration between the anti-Wolbachia consortium and AstraZeneca has identified 14 novel areas of diverse chemical space with potential for ongoing development into treatments for lymphatic filariasis and onchocerciasis. This was accomplished through the first industrial scale screening of a 1.3 million compound collection housed by AstraZeneca against Wolbachia, the A·WOL drug target and endosymbiotic bacteria of the filarial worms which cause onchocerciasis and lymphatic filariasis.

Following a primary screen at 10μM in a model insect cell line (C6/36) stably infected with Wolbachia we obtained ~20,000 hits which were triaged through a chemoinformatic analysis to select 6,000 compounds for secondary dose response screening. Again chemoinformatics were used to cluster the resultant hits into 58 distinct areas of chemical space. Representatives from all 58 clusters were then screened at 5μM in a microfilarial (larval worm) assay. From these representatives 14 clusters demonstrated equivalent Wolbachia reduction to our gold standard drug doxycycline after 6 days *in vitro* exposure. These novel chemotypes, including one related to known oxazolidinone antibiotics, are extremely promising new leads against lymphatic filariasis and onchocerciasis, diseases which afflict 157 million people worldwide resulting in severe disability globally.

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THAT FEELING OF BEING OUT OF PLACE: A MICROFILARIAL TALE

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In, Alberta, Canada, a 27-year-old man presented to the Emergency Department with a complaint of pruritic swelling on his chest. His past medical history was unremarkable. He was born and raised on a farm in Sudan, and drank water from the river. At the age of 12, he fled to Kenya with his family, where he lived in a refugee camp, worked in construction, ate the food provided and drank water from a well. He immigrated to Canada in 2006 and has not returned to Africa since. His symptoms started in January 2016, when he noticed skin eruptions to his right chest. These eruptions were painful, pruritic and at times felt like slowly spreading movement underneath his skin. He presented to a local emergency department where he underwent a minor surgical procedure resulting in removal of a worm from the chest wall lesion. The worm was submitted to the laboratory for identification. Ten days later, the patient felt similar symptoms over his right eyelid; he returned to the hospital for removal of what turned out to be a second worm. He was referred to the Infectious Diseases Clinic. He was completely asymptomatic except for night sweats. His physical examination was unremarkable. There were no ocular findings and no serpiginous lesions on skin examination. Both worms were referred to US Centers for Disease Control and Prevention (CDC) for identification. Blood smear and stool were received in our laboratory; parasitic serology was referred to the National Centre for Parasitology (McGill). The patient was found to have a microfilarial load of >=8,000 microfilariae/ml. Coincidentally, his stool examination revealed rhabditiform larvae of Strongyloides stercoralis . His serology was negative for HIV and positive for Strongyloides sp. and schistosomiaisis (the latter possibly representing a cross-reaction or past exposure). *Loa loa* can live up to 17 years and is not considered endemic in Kenya. Most likely the patient acquired it while in Sudan. This case reports an unusual presentation of loiasis, further complicated by co-infection with S. stercoralis. It emphasizes the importance of obtaining a detailed travel history reaching far beyond the customary few years.

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ELIMINATION OF ONCHOCERCIASIS WITH IVERMECTIN: A VALIDATION OF THE EPIONCHO AND ONCHOSIM MODELS USING DATA FROM MALI AND SENEGAL

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EPIONCHO and ONCHOSIM are two independently developed models for the transmission and control of onchocerciasis. Both models have been used to explore the feasibility of eliminating onchocerciasis from Africa within the timeframes outlined by the World Health Organization (WHO) and endorsed by the 2012 London Declaration on Neglected Tropical Diseases. A recent comparison of projected timeframes to elimination by mass treatment with ivermectin highlighted similarities but also discrepancies between these two models that warranted further investigation and subsequent model refinement. Here we describe refinements to and re-fit the model to parasitological data from human populations in Cameroon and Ghana. We compare the refined version of EPIONCHO and ONCHOSIM in their ability to predict trends in infection prevalence from baseline to elimination in the three West African transmission foci in Mali and Senegal where infection was successfully eliminated circa 2007-2009. We also compare projected timeframes to elimination in terms of programmatic prevalence thresholds, stochastic fade-out and transmission breakpoints and evaluate the impact of uncertainty in key epidemiological and programmatic parameters. We conclude that both EPIONCHO and ONCHOSIM capture epidemiological trends towards elimination with sufficient accuracy to serve as useful decision-support tools for estimating when elimination is likely to be achieved in a variety of epidemiological and programmatic settings and for identifying foci where alternative intervention strategies might be required to reach the WHO targets.

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INTEGRATED LYMPHATIC FILARIASIS AND PODOCONIOSIS CLINICAL CASE MAPPING USING SMS MHEALTH TOOLS AND COMMUNITY NETWORKS IN HAWELLA TULA AND BENSA DISTRICTS OF ETHIOPIA

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Lymphatic filariasis (LF) and podoconiosis are disabling neglected tropical diseases (NTDs) that affect the world's poorest people and pose a significant economic burden. In Ethiopia, an integrated mapping project confirmed that 112 districts are LF-endemic, 345 districts podoconiosis-endemic and 53 districts are co-endemic for both diseases, however limited information is available on the number of clinical cases. Such information is crucial for the effective planning and delivery of a basic package of care. The aim of this study was to identify cases using a bespoke SMS reporting tool, MeasureSMS, in two co-endemic districts, Bensa and Hawella Tula (population 430,439), of the Southern Nations, Nationalities, and Peoples' (SNNP) Region. In July 2015, a total of 59 Health Extension Workers (HEWs) and four district supervisors were trained on how to identify lymphoedema and hydrocele cases in their catchment area (including multiple kebeles) and to report the information by SMS using their own, basic mobile phones. This data was then sent to a local

smartphone with the MeasureSMS app installed, and uploaded to a cloud server where the automatically collated data was accessible via a web browser. A total of 2,197 lymphoedema cases and 134 hydrocele cases were reported, with 42 cases reported as having both conditions. The mean age of all cases was 45.2 years, with 45.4% of lymphoedema and 100% of hydrocele cases being males. All HEWs reported cases with a mean of 40 cases per HEW, with a range of 3 to 210 cases across their multiple kebeles. To ensure data quality, 129 patients were randomly selected to be visited and verified by a trained clinician; 79.1% were confirmed as having the same condition as reported through SMS by the HEW. Network problems and power outages lasting up to two days were some of the challenges faced by the HEWs when using the mHealth tool in the field. Nevertheless, given the high numbers of cases reported in these districts, particularly of lymphoedema, MeasureSMS was shown to be a valuable mHealth tool to obtain patient estimates and guide the allocation of limited resources to those most in need.

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SCALING-UP A 'PACKAGE OF CARE' FOR LYMPHATIC FILARIASIS CASES IN MALAWI USING SMS MHEALTH TOOLS FOR MORBIDITY MAPPING, AND COMMUNITY HEALTH WORKER NETWORKS AND DISTRICT HOSPITALS FOR PATIENT CARE

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After six successful rounds of mass drug administration (MDA) for lymphatic filariasis (LF), MDA in Malawi was stopped in 2014 following nationwide Transmission Assessment Survey (TAS) results. With evident success in interrupting transmission, Malawi, with partner support is now focussed on morbidity management and disability prevention (MMDP) for those affected by clinical manifestations, namely lymphoedema and hydrocele. To begin to scale-up MMDP, three key activities have been implemented in the two most endemic districts, Chikwawa and Nsanje in the Southern Region, and planned in a third district, Karonga in the Northern Region. Firstly, case estimates were obtained using the SMS reporting tool, MeasureSMS, which is an efficient and cost-effective tool by which community health workers (CHWS) identify and report clinical cases of LF in their communities by SMS to develop a clinical database of cases. To then provide access to care for these identified cases, lymphoedema management training of CHWs and hydrocele camps were organised. In Chikwawa district, 369 lymphoedema cases (71 % female) and 986 hydrocele cases were identified by SMS with 11 cases reporting both hydrocele and lymphoedema. In Nsanje district, 265 cases of lymphoedema (77% female) and 866 cases of hydrocele were reported with 9 cases reporting both conditions. To date, 123 of the hydrocele cases have been visited and verified by a clinical to ensure data quality and accuracy. Of these, 99 (80.5%) were confirmed to have the same condition as was reported by the CHWs. In December 2015, 326 of these reported cases had their hydrocele operated at six hydrocele camps across Nsanje and Chikwawa districts with impact assessments conducted to demonstrate an improvement in quality of life. In total, 468 CHWs were trained on lymphoedema management (integrated with leprosy) and hydrocele referral in early 2016. These results demonstrate that Malawi has made significant progress in scaling up MMDP activities and will continue to move these activities forward with a commitment from the Ministry of Health and partners with the aim of meeting the 2020 global and national elimination goal.

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LESSONS FROM MASS DRUG ADMINISTRATION FOR THE ELIMINATION OF LYMPHATIC FILARIASIS (LF) IN AN URBAN SETTING IN HAITI

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The world is increasingly urbanized as 54% of the world's population lives in urban areas, including 57% of the population in Haiti. Urbanization has far-reaching health consequences, as these highly mobile and heterogeneous populations have varying experiences and perceptions of the health system. As lymphatic filariasis (LF) is endemic throughout Haiti, the NTD Control Program has a goal to eliminate LF as a public health problem by 2020 through mass drug administration (MDA). To achieve elimination, at least five rounds of MDA with ≥ 65% coverage must be completed for the population at risk. High coverage rates are necessary for MDA to effectively reduce LF prevalence to a level at which transmission is not sustainable. Peri-urban settings in Haiti typically require additional rounds of MDA. Two communes were selected, the peri-urban Croix-des-Bouquets and rural Thomazeau in the West department for a Knowledge, Attitudes and Practice (KAP)/coverage survey supported through USAID's ENVISION Project. A two-stage 30-cluster sample was used to ensure random selection. Data collection took 7 days and analyzed with STATA version 14. The survey coverage for Croix-des-Bouquets was 53.3% compared to reported coverage of 67.09%; 80% for Thomazeau (61.3% reported coverage). The peri-urban nature of Croix-des-Bouquets may influence how and why respondents participate in MDA. Croix-des-Bouquets is of interest as Haiti nears LF elimination, since success relies on high coverage urban MDAs. When asked how they heard about the MDA campaign, the most popular response was megaphones (35% Croix-des-Bouquets and 65% in Thomazeau). The survey also found that population movement and general mistrust of the health system and participating in projects supported by international aid, contribute to low coverage rates. Further, those in urban settings have access to a greater variety of media therefore diluting the effectiveness of MDA messaging. Focusing on more direct ways to reach the population and alternative social mobilization strategies tailored to specific needs of the urban community may help overcome these barriers.

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LESSONS FROM LYMPHATIC FILARIASIS TRANSMISSION INTERRUPTION IN HAITI: ARE FIVE ROUNDS OF ANNUAL MASS DRUG ADMINISTRATION (MDA) NECESSARY IN LOW-PREVALENCE SETTINGS?

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Lymphatic filariasis (LF) is endemic throughout Haiti. In line with World Health Organization's (WHO) LF elimination targets, the Haiti NTD Control Program (HNTDCP) has a goal to eliminate LF as a public health problem

by 2020. Currently WHO recommends 5 rounds of consecutive annual mass drug administration (MDA) with ≥65% coverage, after which a transmission assessment survey (TAS) can be carried out to determine whether MDA can be stopped. Ile de la Tortue, a commune in the Northwest department, is a remote island that had an antigen prevalence of 6.0% in 2000 and only 0.8% by 2006 sentinel site evaluation. Two rounds of MDA were conducted in Ile de la Tortue 2003 and 2005 with 110% and 87% reported coverage, respectively. MDA was then suspended due to resource constraints. In 2012, the HNTDCP with assistance from CDC and UND carried out TAS1 in order to determine if additional rounds of MDA were necessary to reduce antigen prevalence to below 2%. School-based TAS was carried out on a sample of 1,308 11-12 year olds, with 13 immunochromatographic card test (ICT) positive cases, which was below the calculated critical cut-off for sample size of 14. This older age group was used because school enrollment for 6-7 year-olds was significantly below the recommended 75%, and enrollment did not exceed 75% until the 11-12 age group. Further, using an older age group is appropriate in settings where MDA has not been recently carried out. In 2016, the HNTDCP and IMA World Health/ENVISION carried out a school-based TAS2 in Ile de la Tortue. A total of 928 6-7 year olds were tested, and 0 were ICT-positive. These results indicate that 12 years since the last treatment, there is no evidence of ongoing LF transmission and additional MDA is not indicated. The results suggest that 5 rounds of annual consecutive MDA may not be necessary in areas with adequate MDA coverage and low baseline prevalence. Further research is warranted to determine the number of rounds of MDA required, as reducing these could help resource-poor settings with low initial prevalence reach their 2020 LF elimination goals.

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PROJECTIONS OF ATTAINING ONCHOCERCIASIS ELIMINATION IN OGUN STATE, NIGERIA: A CROSS-SECTIONAL REPORT OF THE OV-16 SEROLOGY (RAPID DIAGNOSTIC TEST) AMONG CHILDREN BORN AFTER 10 YEARS OF TREATMENT WITH IVERMECTIN

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Evidence based studies using the Ov-16 serology among children below 10 years of age is one of the key components of the revised WHO course of action for indicating interruption of transmission of *Onchocerca volvulus* among human population receiving treatment with ivermectin. In view of this background, this study conducted between March and July 2015 investigated the sero-prevalence of onchocerciasis in endemic communities of Ogun State, Nigeria after 10 years of treatment with ivermectin. Using the Ov16 Rapid Diagnostic Test (RDT), 719 children between the age 5-9 years residing in 32 firstline communities in 8 endemic Local Government Areas (LGA's) provided whole blood specimen which were tested for IgG4 antibodies against the *O. volvulus* antigen Ov-16. Data were analysed using Pearson's Chi square in SPSS 20. Results showed a cumulative sero-prevalence of 21(2.9%), Relationship between age and prevalence was statistically insignificant ($p > 0.05$). Thirteen females and eight males were exposed to *O. volvulus* respectively. The low sero-prevalence recorded among children within the age range (5-9 years) born after the inception of ivermectin implementation implies that they may have had diminutive historic exposure. Although this finding is somewhat greater than the 0.1% threshold set by the guideline for this study population. The Information obtained will serve as a baseline serological information and a guide to prepare Ogun State for Post Treatment Surveillance (PTS) in the nearest future.

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INTEGRATING NOVEL PEPTIDES AND REPORTER NANOPARTICLES IN A RAPID TEST FOR ONCHOCERCIASIS

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This study describes a lateral flow assay (LFA) to detect exposure to *Onchocerca volvulus*. The assay features two innovations: a novel peptide biomarker and novel nanoparticles. The advent of high throughput peptide synthesis and micro-array technology enables new avenues for the discovery of diagnostic targets. Scanning the *O. volvulus* proteome for immunodominant linear epitopes revealed at least three highly immunogenic motifs, as reported previously. In parallel, we have introduced new plasmonic reporter nanoparticles to increase the analytical sensitivity of LFAs. These nanoparticles are gold shells, consisting of a gold layer deposited over a low-density silica gel core. The shells are blue to black and absorb visible light 35 times more efficiently than the red colloidal gold typically employed in LFAs. The increase in absorption translates into a stronger visual readout and an increase in analytical sensitivity. The two technologies were merged into a prototype LFA that employs gold shells to detect circulating antibodies to one of the recently discovered peptide motifs. A total of 20 plasma samples (FR3 repository) from individuals suffering from onchocerciasis and 16 samples from non-endemic healthy controls were tested i) in the Ov16 LFA, ii) in a peptide ELISA, and iii) in the peptide LFA using gold shells. The Ov16 LFA showed 85% and 100% sensitivity and specificity, respectively. The peptide ELISA gave 90% sensitivity and 100% specificity. The peptide LFA was 80% sensitive and 100% specific. The peptide LFA results correlated with the peptide ELISA data, and the four samples that scored negative in the LFA gave no or very low signal in the ELISA. Onchocerciasis samples lacking Ov16 antibodies did recognize the peptide in ELISA and LFA, and conversely, samples lacking antibodies against the peptide reacted in the Ov16 LFA. Thus, a peptide LFA was assembled which, when used in combination with the Ov16 LFA, and when tested on a small sample collection, detects antibodies to *O. volvulus* with a sensitivity of 100% and specificity of 100%.

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IMMUNOREACTIVITY OF AN ON*CHOCERCA VOLVULUS* LINEAR EPITOPE IN INDIVIDUALS FROM DIFFERENT REGIONS IN GHANA

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Using proteome-wide high-density peptide microarrays we have identified an immunodominant linear epitope, as reported previously. We have used peptide ELISA to assess the immunoreactivity of a 15-mer peptide 02-052 containing the identified motif 2, in individuals from Ghana. Plasma samples were collected from nodule-positive individuals (n=94), endemic controls (n=48), non-endemic controls (n=20) and lymphatic filariasis patients (n=45). A healthy control group of 10 samples from South Africa was included to assess specificity. All samples were tested on the Ov16 IgG4 lateral flow test (LFA) (Standard Diagnostics). None of healthy controls from South-Africa and non-endemic controls were positive in this test, while 24/48 endemic controls, 68/94 nodule-positive, and 12/45 LF patients were positive for Ov16 IgG4 antibodies. 'No peptide' controls were included for all samples to assess non-specific binding of antibodies (i.e. background). A cut-off for positivity was set at the average background + 3SD. None of the healthy control samples had detectable antibodies against the peptide. In the non-endemic control group, 3/20

were positive in the peptide ELISA. In the endemic control group, 17/24 and 14/24 of the Ov16 positive and negative individuals, respectively, were positive in the peptide ELISA. 40/68 of the Ov16 positive nodule-positive individuals, and 13/26 of the Ov16 negative nodule-positive individuals, had detectable antibodies against the peptide. Also in the LF group, 8/12 and 23/33 of the Ov16 positive and negative individuals, respectively, were positive in the peptide ELISA. In conclusion, the new *O. volvulus* linear epitope showed perfect specificity in healthy controls from South Africa. This peptide ELISA identifies more cases of exposed/infected individuals as compared to the Ov16 IgG4 test. Reactivity in LF patients residing in onchocerca endemic areas requires in depth analysis and will drive the use case for peptide LFA. This peptide has been used previously for development of a LFA.

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DEWORMING IN PRE-SCHOOL AGE CHILDREN IN NIGERIA: ARE THOSE WHO NEED IT THE MOST RECEIVING TREATMENT?

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Pre-school age children are at high risk for nutritional and growth impairments from soil transmitted helminths (STH) and yet, despite the World Health Organization's recommendation of periodic treatment for those in that age group in endemic areas, they are currently not receiving the deserved attention from national control programs. We examined the deworming status of pre-school aged children in 12 high prevalence states in Nigeria and whether those at increased risk of infection or with related symptoms received treatment. Children aged 12-59 months were selected from the 2013 Nigeria Demographic and Health Survey, a national representative sample survey of adults aged 15-49 years. Markers of risk for infection with STH were state prevalence levels (≤ 20%), socioeconomic status and other demographic variables. Symptomology was represented by the presence of stunting. Weighted logistic regression was used to determine the association of infection risk factors and stunting with mother's reports of whether children received treatment. Of the 3,062 children considered in the analysis, 39% received treatment for worm infections. Compared to children with normal height, severely and moderately stunted children were less likely to access treatment with OR=0.45 (95CI: 0.31 - 0.64) and OR =0.69 (95CI: 0.51 - 0.93) respectively. Uneducated mothers and fathers were less likely to report treating their children with OR = 0.52 (95CI%: 0.35 - 0.75) for mothers and OR =0.42 (95CI%: 0.25 - 0.72) for fathers. Poorer children were less likely to access treatment than the richest children with OR = 0.41 (95CI%: 0.26 - 0.64). The disparities in treatment access among stunted children as well as the potential influence of socio-economic factors call for increased need for deworming coverage of pre-school aged children in Nigeria. Deworming interventions can prevent children from becoming more stunted and may even reverse it. Control programs must identify the most-at-risk populations in order to leverage existing resources to decrease intestinal worm prevalence and break transmission.

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SITUATIONAL ANALYSIS OF NEGLECTED TROPICAL DISEASES MANAGEMENT INFORMATION SYSTEM IN ETHIOPIA

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Health Management Information System (HMIS) is critical for neglected tropical diseases (NTDs) services delivery and informed decision making. In Ethiopia the current situation of the NTD-HMIS is not clearly known. This study is therefore conducted to assess the existing NTD management information system and thereby generate evidence for formulating interventions for improving the system in Ethiopia. The situational analysis involved collection of both quantitative and qualitative data using structured self-administered questionnaire, observation and key informant interview among district monitoring and evaluation officers, district managers, health extension workers and regional NTD program managers. Data was analyzed by using Epi-Info version 3.5.4 and descriptive statistics was conducted using the software. A total of 11 NTD endemic regions in Ethiopia were included in this study. Of these, the largest proportions (66.6%) had no NTD team at regional level, thus the program was executed by focal person. About a third (33.1%) of respondent in the sample had not received a training workshop or technical briefing on monitoring & evaluation of NTDs. According to the NTD data management, it was reported that (83.3%) of the respondent were using simple Excel file to store their data, and the remaining (16.7%) were using paper based mechanism to keep. Regarding data quality assurance, all of the regions (100%) had not carried out data quality assessment. Findings for the NTD medicine supply and management revealed that most of the region (83.3%) had a system for tracking medicines remaining in stock and wastage in the region while only (16.7%) of the overall sampled had no tracking system. This study recommends that collaboration among the stakeholders on capacity buildings of management information system, supportive supervisions with timely and concrete feed backs, and establishment of functional NTD monitoring and evaluation working group at regional level is encouraged. Continual presence of standardized data collection tools and use of information technology for management information system should be given due attention.

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TREATMENT COVERAGE VALIDATION SURVEY AFTER A SCHOOL-BASED MASS DRUG DISTRIBUTION OF PRAZIQUANTEL AND MEBENDAZOLE IN SELECTED DISTRICTS OF ETHIOPIA

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The Ethiopian Ministry of Health is scaling up programs to deliver regular, large-scale schistosomiasis (SCH) and soil-transmitted helminths (STH) treatment for at-risk populations, through repeated rounds of preventative chemotherapy (PCT) using Praziquantel (PZQ) and Mebendazole (MEB). One of the key indicators of program success is program coverage. The aim the surveys presented here is to produce validated coverage estimates of reasonable precision following mass drug administration round in April 2015 in randomly selected district. To assess the coverage of mass drug administration in the dewormed districts of Ethiopia. A community-based cross-sectional study design was employed. Accordingly, 960 households from 96 kebeles found in the sampled 8 districts were selected. The survey covered districts in Amhara, Oromia and Southern Nations Nationalities and Peoples Region. Participation in the treatment campaign and other related Information was collected from school age children (SAC) between the age ranges of 5 to 14 years. 960 households were selected for the validation survey and a total of 1910 (97.2%) school age children (SAC) residing in the selected households between the age ranges of 5 to 14 years were interviewed. During the house hold visit, 55(2.8%) households were not available for interview. While 81.2% of the school age children interviewed were attending school, 18.8% were non-enrolled. 91% of the interviewed children were present in schools during the mass drug administration campaign. During the treatment campaign 84.9% and 85.3% interviewed SACs swallowed Praziquantel and Mebendazole respectively and there was no difference by gender. Our current finding shows that approximately 85% of school age children interviewed received the treatment and the coverage is very high in enrolled school age children. Based on these findings, we recommend creating mechanisms to

reach non-enrolled school age children and awareness in the community if targeting 2020 to control both schistosomiasis and soil-transmitted helminths in Ethiopia.

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TO INTEGRATE OR NOT TO INTEGRATE? DEVELOPING AN EVIDENCE-BASED TOOL FOR NEGLECTED TROPICAL DISEASE CONTROL

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While evidence from clinical and modeling studies have demonstrated the potential benefit of integrating vertical disease programs, few evidence-based tools exist to assist decision makers in evaluating and comparing approaches for integration of control measures, to determine the most impactful and cost-effective approaches for their setting. We have created an application available for mobile devices or on the web, with a simple user interface, to support on-the-ground decision-making for integrating disease control programs or their components, given local conditions and practical constraints. The model upon which the tool is built provides predictive analysis for the effectiveness of integration of schistosomiasis and malaria control, two common parasitic diseases with extensive geographical and epidemiological overlap, and which result in significant morbidity and mortality in affected regions. Working with data from countries across sub-Saharan Africa and the Middle East, we present here a proof of principle for the use of our tool in providing guidance on how to optimize integration of vertical disease control programs.

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SURGICAL MANAGEMENT OF MORBIDITY DUE TO LYMPHATIC FILARIASIS: HYDROCELE SURGERY IN HEALTH DISTRICT HOSPITALS IN MALI

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Mali is committed to national lymphatic filariasis (LF) elimination by 2020. The program has two main components: interrupting transmission of LF through mass drug administration and managing morbidity and preventing disability. Mali has achieved great progress toward LF elimination: to date, 31 of the 63 health districts (HDs) have reached the criteria to stop MDA. However, improvements in morbidity management and disability prevention are more difficult to achieve. Interventions for the surgical management of hydrocele and lymphedema management were introduced in 2012. These interventions are very demanding by the patients because of social impacts in their daily live. From 2014-15, the national program, with the support of HKI with funding from the End Fund, performed surgical treatment of 369 LF patients in 16 HDs. General practitioners performing surgery were trained on hydrocele case management and hydrocele cases were identified at the community level. Surgeons obtained informed consent prior to the surgical procedure and maintained patient records. A descriptive analysis to show the impact of hydrocele management was performed on 175 patients who were included in this analysis based on the completeness of the patient record. The median age of patients was 52 years and 75% (132/175) were married. The majority of patients 82.3% (144/175) did not report postoperative complications. The median duration of hospitalization was 4 days (ranging 2-55 days). Results showed that 32.6% (57/175) patients reported a considerable positive impact on their work and 44.6% (78/175) reported improved sex lives. Of those patients operated, 77% (54/70) indicated satisfaction after surgery and noted improvement in their daily lives. Dissatisfied patients had developed postoperative secondary infections that were managed with supportive therapy. This study shows that the management of hydrocele surgery can be done in a district hospital setting in Mali without major complications and achieve general patient satisfaction. Consequently, the LF morbidity management and disability prevention project will be expanded to other HDs.

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ACHIEVING THE ENDGAME: IMPROVING INTEGRATED CASE SEARCHES FOR GUINEA WORM DISEASE AND TRACHOMA TO ACHIEVE ERADICATION AND ELIMINATION TARGETS

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Guinea worm disease and trachoma are both neglected tropical diseases (NTDs) slated for eradication and elimination as a public health problem, respectively, by the World Health Organization. In 2010, The Carter Center, the Ghana Ministry of Health and local partners, carried out integrated case searches for rumors of possible cases of Guinea worm disease and persons with trachomatous trichiasis (TT), the end stage of trachoma. These case searches were conducted to meet eradication and elimination targets. The first series of case searches were carried out in four districts, which were searched community to community, with patients referred to a centralized surgical facility. This method of searching did not adequately cover the target population and resulted in low surgical uptake. The second series of case searches, which were conducted house-to-house with patients being offered immediate investigation of suspected Guinea worm disease cases and TT surgical care either in the home or an adjacent primary care facility. This method resulted in higher surgical uptake. The house to house immediate resolution approach was also shown to be more cost effective. The cost to investigate suspected cases of both diseases in the house to house immediate response approach was about USD$13.99 per case examined, compared to a cost of $19.78 per case examined in the community with referral to surgical facilities approach. A review of the two approaches showed significant cost differences and favorable outcomes to the house to house immediate response approach. This approach should be considered an option for disease "end game" where case searches are required to meet targets. This approach should be considered in an integration fashion with other NTDs to maximize cost saving and efficient use of resources.

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ARE WE REACHING EVERYONE AS WE MOVE FROM CONTROL TO ELIMINATION OF NTDS: FINDINGS FROM AN INTEGRATED TREATMENT COVERAGE SURVEY IN NORTHERN NIGERIA

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Countries like Nigeria have developed and are implementing programmes for integrated control of Neglected Tropical Diseases (NTDs). Sightsavers is leading consortium of partners in scale-up for elimination of NTDs in northern Nigeria. However, little data are available to verify reported coverages of previously conducted Mass Drug Administration (MDA). The purpose of this presentation is to validate the reported coverage from Community Drug Distributors (CDD) through integrated post-MDA

coverage survey in Kaduna, Katsina and Niger states. This also highlights differences by drug type, persons with disabilities (PWD) and reasons for non-compliance. A population based survey using two-stage cluster sampling methods was conducted to verify the proportion of individuals who ingested the drugs during last rounds of MDA. Washington Group (WG) questions were integrated into the questionnaire. Since not all drugs were distributed at the same time, to reduce recall bias, drug samples were shown to participants during the survey. A total of 7,688 persons were enrolled from six Local Government Arears (LGAs) in three states. Overall, the therapeutic coverages in survey population were lower than those reported by CDDs. 58.6% (95% CI 56.0-61.2) and 68.7% (95% CI 66.1-71.3) swallowed Mectizan/Albendazole in Kubau and Soba LGAs of Kaduna state respectively. Two LGAs in Katsina had coverage of 54.9% and 50.4% (95% CI 52.3-57.4 and 47.6-52.9) while the reported coverage was 18% and 102% respectively. Niger had survey coverage of 66.2% (95% CI 64.9-70.4) in one LGA while they reported 32%. Large proportions of eligible persons with severe disability were missed during MDA for all drugs. Among eligible population in Kaduna, 3.5% of PWD reported ingesting Mectizan/Albendazole, while 96.5% were non-disabled. Similar lower coverages were seen in other two states. Despite the intervention being community driven, more efforts are required to ensure adequate and equitable coverages are reached, and maintained at WHO levels for elimination to be attained. These reported levels should be verified so that appropriate measures are taken to improve coverage.

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THE IMPACT OF MASS DRUG ADMINISTRATION ON HOOKWORM AND SCHISTOSOMIASIS IN LOFA COUNTY, LIBERIA BEFORE AND AFTER THE OUTBREAK OF EBOLA VIRUS DISEASE

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Lofa County was an epicenter of the 2014 Ebola virus disease (EVD) outbreak, and an extensive hand washing and hygiene campaign was launched in the summer of 2014 that lasted about 18 months. We are comparing the impact of annual (1x per year) vs semiannual (2x) mass drug administration (MDA) with ivermectin, albendazole and praziquantel on lymphatic filariasis, onchocerciasis, soil transmitted helminths (STH), and schistosomiasis in 32 villages. This study focuses on results for STH and schistosomiasis. Between 1,850 and 2,678 subjects aged 5 years and older were screened each year by duplicate Kato Katz stool exams. Rates and intensities of ascariasis and trichuriasis were low at baseline in 2012, but hookworm and *Schistosoma mansoni* infections were rampant with prevalence rates of 61% and 88% and geometric mean egg counts of 232 and 213 epg, respectively. Prevalence rates in the spring of 2014 following MDA were essentially unchanged, but egg counts for hookworm and *S. mansoni* were reduced from baseline by 51% and 31%, respectively. Ebola prematurely ended our survey in 2014 and prevented MDA for 1 year, but the epidemic brought extensive changes in hygiene in the study communities. MDA was reintroduced in April 2015, and the communities were re-tested in the spring of 2016. At that time the hookworm prevalence rate was only 27% (a 45% reduction from 2014), and with only 47 epg (a 59% reduction from 2014). In contrast, the reductions in *S. mansoni* prevalence (10%) and intensity (20%) during this time interval were unimpressive. These results suggest that local elimination of hookworm may be easier to achieve with MDA than local elimination of schistosomiasis. We also suspect that the dramatic reduction in hookworm after the EVD outbreak was due to improved hygienic practices that reduced reinfection rates following MDA. The EVD outbreak provided a unique opportunity to study the additive impact of hygiene on top of MDA.

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FINDINGS FROM A SITUATIONAL ANALYSIS FOR INTEGRATED COMMUNITY CASE MANAGEMENT IN RURAL HEALTH ZONES OF HAUT-KATANGA IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Though rich with resources, the population of Katanga province in the Democratic Republic of Congo (DRC) is primarily rural, poor and harbors some of the highest child mortality rates in country. The province also has the lowest ratio of health care workers (HCWs) to inhabitants, particularly nurses (4 per 10,000 persons), and health coverage is very low: only 25% have access to health facilities (HFs) within 5 km of distance. In response, the DRC recently developed guidelines for implementation of integrated community case management (iCCM). In response, the MalariaCare partnership assessed nine health zones (HZs) in Katanga for opportunities to develop community health sites (CHS). Following national criteria - which include parameters for population density and preference for higher difficulty of access to and >5km distance from the local health center - the project found 96 eligible villages in 52 health areas (HAs) during a pre-visit screen. Following on-site assessments, MalariaCare further modified selection criteria to only include sites >10km & <30km from health center with head nurses able to supervise CHS. A final group of 53 villages in 45 HAs were ultimately identified for CHS development. Several challenges were encountered during this site selection process. For example, the average population per village site is 2,389 persons (median population is 2,003; range 912-7,400), exceeding the recommended 1,000 persons (500 per community health worker—CHW) per CHS. Additionally, the average distance between a CHS and an HF was 17.3 km [5.6-48 km], with a median of 15 km - raising challenges for adequate supervision and adequate resupply. This mapping exercise demonstrated that using reasonable CHS criteria, widespan implementation of CHW-based iCCM in this province will be challenging due to the lack of supporting health facility infrastructure. Consequently, supervision models should be developed to allow for the higher population densities and longer distances from health facilities, than were originally anticipated for implementation of iCCM in Katanga province.

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DEVELOPING EVIDENCE BASED COMMUNICATION AND SOCIAL MOBILIZATION STRATEGIES 26MASS DRUG ADMINISTRATION

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There is a need for stronger, evidence based, social mobilization approaches and information education and communication (IEC) materials to support behavior change for mass drug administrations (MDA) in the control of neglected tropical diseases (NTDs). In order to address this problem a draft guide on how to design effective social mobilization strategies and IEC materials for MDAs was developed. Results of the study that served to guide the development of this document are presented here. The study consisted of a desk review and four rapid case studies - from national NTD programs supported by ENVISION, funded by the

U.S. Agency for International Development (USAID) and managed by RTI International. Case studies included a budget and expenditure analysis of IEC and social mobilization budget lines, information extracted from knowledge attitudes and practices (KAP) surveys, review of IEC and social mobilization materials and strategies, and in-depth interviews with key informants. The total dollar amount spent on IEC and social mobilization as a percentage of total program costs ranged from 4.2% to 11.8%. The items that account for the highest portion of this budget were print, wearable items, events, town criers and radio, but this varied considerably by county. Practices identified with potential for success across different country settings included use of community distributors as trusted sources of information and inclusion of messages focused on side-effects. Opportunities for strengthening social mobilization included: revise IEC materials that were often too technical and lacked information related to taking part in MDAs, strengthen social mobilization and communication in training of drug distributors, develop simple strategies based on evidence, and evaluate materials and strategies. The resulting guide, including templates and planning tools, is briefly presented.

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A COMPREHENSIVE SUSTAINABILITY FRAMEWORK FOR NEGLECTED TROPICAL DISEASES ELIMINATION PROGRAMS

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Neglected tropical diseases (NTDs) are a group of 17 parasitic and bacterial infections that cause disability and kill more than 500,000 people a year worldwide (Hotez, 2008). The World Health Organization (WHO), the world's leading authority on health issues, has declared at least 10 of these NTDs as controllable and in some cases eliminable (WHO, 2014). Lymphatic Filariasis (LF) and onchocerciasis are two of the NTDs that are targets for elimination by 2020 and 2025 respectively (WHO, 2014). In order to reach the elimination goals, WHO has overseen the development of more than 74 country specific multi-year national plans for the control and elimination of NTDs (NTD Master Plans). These plans provide the framework for countries to start elimination programs, rapidly scale the programs up to reach all those in need of treatment, and to sustain treatment for the needed duration and initiate long-term environmental changes that prevent re-infection, as reported previosly. Sustainability research however, reveals that the approaches recommended by WHO do not address all the necessary steps that NTD programs must take in order to sustain program activities and outcomes to ensure disease elimination. More specifically, while the plans provide the necessary technical guidance to reach control or elimination, they lack adequate guidance on how to address non-technical aspects of sustaining the programs beyond suggesting the strengthening of government ownership, enhancing planning for financial sustainability and integrating control of NTDs into national primary health care systems, as reported previously. Using multi-case study methodology, this qualitative research adopts available sustainability frameworks in health and development sectors to LF and onchocerciasis elimination programs in two states, four local government areas in Nigeria. The result is a sustainability framework that defines the critical components of NTD programs that need to be sustained and the means by which each of these components can be sustained.

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THE ROAD TO ELIMINATION OF SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTHS AS PUBLIC HEALTH PROBLEMS: THE MALAWI STORY SO FAR

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Since 2012, the Malawi Ministry of Health (MoH) with technical support of the Schistosomiasis Control Initiative, have successfully carried out four national preventive chemotherapy (PC) campaigns totalling 14.6 million treatments with praziquantel and albendazole to reduce schistosomiasis (SCH) and soil transmitted helminth infections (STH). Impact surveys have shown the Malawi programme is at a critical stage on the road to elimination of SCH and STH as public health problems. Results from WHO recommended monitoring and evaluation activities to assess a program by measuring impact, performance and process are combined to illustrate the programmes achievements. For impact evaluation, twenty-two sentinel schools were randomly sampled for data collection in approximately 2,500 school-age children, prior to each national PC campaign. Cross-sectional parasitological data was used to evaluate prevalence and high-intensity infection of Schistosomiasis and STH. To validate programme performance in terms of treatment coverage, multi-stage cluster surveys were used. The first conducted after the 2012 treatment campaign, with subsequent surveys carried out in 2014 and 2016. A data quality assessment (DQA) study allowed the Malawi programme to review the effectiveness and efficiency of the MoH reporting systems for treatment numbers. Despite numerous challenges, reductions in prevalence and high-intensity infection have been observed for both Schistosoma species and STH in all target groups. Results from the coverage surveys have shown the need to increase treatment coverage of non-attending school-age children and adults in areas known for high transmission. Finally the DQA survey has highlighted weaknesses in reporting process which the programme has now adapted. The results of the surveys will be presented and illustrate how subsequent programmatic adaptions to a program in its infancy, in training, communication and delivery, have led to improved efficiency and effectiveness of control efforts. The next steps, taking this program beyond control to an elimination of as a public health problem phase, will be discussed.

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SHRINKING THE NEGLECTED TROPICAL DISEASE MAP IN TANZANIA: TRACHOMA AND LYMPHATIC FILARIASIS

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The Tanzania Neglected Tropical Diseases (NTD) Control Program has made significant progress since its launch in 2009, achieving complete geographic coverage for mass drug administration (MDA) for the 5 preventive chemotherapy (PCT) NTDs including lymphatic filariasis (LF), soil transmitted helminths (STH), onchocerciasis, trachoma and schistosomiasis in 2016. The results of LF transmission assessment surveys to date indicate that transmission in some implementation units has stopped, reducing the number of endemic districts from166 to 103, with LF remapping confirmation in 2015. In 2009, one district met the criteria for stopping MDA; 5 additional in 2014, and 33 additional districts by 2015 making a total of additional 39 districts reaching criteria for stopping MDA. Thus in 2016, only 63 districts will need LF MDA. A total of 56 districts were ever trachoma endemic above the treatment threshold (greater than or equal to 10%). Another 4 districts were endemic with prevalence between 5-9.9% at baseline. The program also completed trachoma mapping nationwide in 2014; only 3 districts (Chunya, Ngara and Chemba) were found to be endemic with above 5% trachomatous inflammation, follicular (TF), thus requiring Zithromax MDA. Impact surveys indicated that trachoma MDA could be stopped in 22 districts, thus further shrinking the Tanzania trachoma map. By the end of 2015 only 18 districts needed Zithromax MDA. Additional districts are expected to achieve the TF level of <5% and meet the WHO criteria to stop MDA in 2016. Tanzania is on track to reach its 2020 trachoma and LF elimination targets, in line with WHO goals provided that the program is able to sustain the scale-down trend in MDA and strengthen other disease control and elimination intervention measures.

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HEALTH-SEEKING BEHAVIOR FOR EPILEPSY IN AN ONCHOCERCIASIS ENDEMIC AREA OF CAMEROON

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Certain onchocerciasis-endemic areas in central Africa, such as Northern Uganda, South Sudan, Tanzania, the Democratic Republic of Congo, and potentially Cameroon are affected by a severely debilitating form of epilepsy called Nodding Syndrome (NS). In certain areas of Cameroon an increased prevalence of epilepsy might constitute a form of NS although diagnosis is unclear as the exact cause of the disease is still unknown. To inform policy on health provision for epilepsy and/or NS, an ethnographic study was carried out on local health seeking itineraries of people severely affected by epilepsy in 5 onchocerciasis-endemic villages in the Sanaga basin area in Cameroon. Patients generally chose between treatment in the biomedical sector, traditional healing or religious services. Treatment choice was mainly influenced by the cost of treatment, accessibility of health providers and perceived aetiology of the disease. The sudden increase of epilepsy over the last 40 years is often attributed to sorcery. As such, epilepsy is believed to be 'thrown at' or 'injected' into people using mystical means. While some people with epilepsy do not attend health facilities due to these aetiological beliefs, most seek a combination of biomedical and traditional care. As biomedical treatment is commonly perceived to have a merely calming effect on the disease, traditional healers are consulted to address the root of the problem, usually referring to increased social tensions or accusations of unnatural acquisition of wealth through sorcery. In terms of biomedical care, once satisfying medication is found, usually no medical follow-up consultations are solicited and additional medicine can be obtained from both official and unofficial sources. Alternatively, a religious path can be chosen, potentially leading to the interruption of biomedical treatment in order to let God cure the disease. Strengthening access to appropriate care is urgently needed as research in similar contexts has shown that even when the disease aetiology is perceived to be sorcery, people will attend biomedical care if this is perceived to improve patients' condition.

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DELIVERING INTEGRATED PREVENTIVE CHEMOTHERAPY EN-MASSE FOR NEGLECTED TROPICAL DISEASES IN TANZANIA

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Neglected tropical diseases (NTD) cause a serious public health problem in sub-Saharan Africa, mostly due to poverty and inadequate sanitation. In Tanzania, lymphatic filariasis, trachoma, onchocerciasis, soil transmitted helminthiasis and schistosomiasis are endemic, putting all 50 million inhabitants at risk of infection. The World Health Organization urges member states to initiate integrated control programs using available highly effective low cost interventions like preventive chemotherapy (PCT). In 2009, Tanzania launched an integrated NTD program that works to deliver mass treatments to affected communities. Program has expanded in phases from a geographic coverage of just 27% in 2010 to 100%- in 2010 for onchocerciasis, in 2014 for LF, and in 2016 for STH and Schistosomiasis. By 2016, the program covers (all) 166 implementation units with required PCT packages. The program is uniquely designed as all interventions are delivered through the central ministry of health's decentralized healthcare delivery system. PCT is planned and implemented at the district level. Treatments are distributed through community and school mechanisms with one reporting system countrywide. This design makes it possible for the program to deliver large numbers PCT per annum. A total of 55 million treatments were delivered to over 22 million people in 2014, and 45 million treatments to over 19 million people in 2013. In 2016, the program aims at delivering 60 million treatments to 23 million people. Key to this success is an army of dedicated medicine distributors; community based drug distributors and primary school teachers. Their involvement increases as the program expands, from 21,902 in 2009 to 113,689 community based drug distributors in 2016 and from 3,065 to 36,985 primary school teachers. They receive training yearly on PCT administration and are supervised by over 14,047 health workers. Over the past 7 years, great success in delivering PCT to large and diverse populations has been registered and Tanzania is close to reaching the targeted end points.

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TANZANIA ON TRACK TOWARDS ACHIEVING GLOBAL GOALS FOR CONTROL AND ELIMINATION OF NTDS BY 2020, EVIDENCE FROM THE FIELD

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The Regional Strategic Plan for NTDs in the African Region 2014-2020 is guided by three key objectives which national NTD programs are working to achieve by 2020. The objectives are to scale up access to interventions and systems capacity strengthening; enhance planning for results, resource mobilization and financial sustainability of National NTD programs; and strengthen advocacy, coordination and national ownership. NTDs are endemic in all parts of Tanzania, with an estimated 47 million people at risk of infection with two or more NTDs. Tanzania's NTD program targets schistosomiasis, trachoma, lymphatic filariasis, onchocerciasis, and soil transmitted helminthiasis through community and school-based mass drug administration (MDA) of preventive chemotherapy. The program evaluated the achievements of the Tanzania NTD control program toward the African Regional Strategic goals for NTD control and elimination for the period 2014-2016. By 2014, the geographical coverage for specific disease preventive chemotherapy was 97.94% for LF, 100% for onchocerciasis, 35.54% for schistosomiasis, 59% for STH and 100% for trachoma. With program expansion geographical coverage rose from 64% in 2014 to 100% by end of 2016. All 26 regions and 166 districts have government employed NTD coordinators and trained NTD secretariats managing NTD activities. Each health facility has staff trained in NTD control program implementation. Advocacy and sensitization for NTD control has reached all senior government and political leaders in all regions and districts. There are pockets of lower MDA coverage rates in hard to reach communities and where the estimated number of school-aged children is not well established. Evaluation results indicate that Tanzania has made progress toward all four African regional strategy key objectives. In order for the program to build on these significant improvements, further steps are needed to address insufficient MDA coverage in some districts, and to address the morbidity caused by these diseases.

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WHILE YEMEN IS HEADING TOWARDS SCHISTOSOMIASIS ELIMINATION, WAS IT SUCCESSFUL IN ENSURING EQUITY?

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The SCH (Schistosomiasis Control Programme) (soil transmitted helminths) STH programme in Yemen has been suspended two times during its cycle (2010-2016). The first was in 2011-2012 due to the involvement of Yemen in the Arab spring that led to a revolution against the former regime, and in 2015- until current due to civil war between several armed militias from one side and an external war with neighbouring countries from the other side. The programme has resumed since February 2016 despite the continuation of war. Meanwhile, over 25 million free treatments (using 62 million tablets of praziquantel and 25 million tablets of albendazole) have been provided to Yemenis living in endemic areas for schistosomiasis and soil transmitted helminths during the years of 2010 -2015. Yemen NTD programme mainly the schistosomiasis and the soil transmitted helminths has reached to a stage that following treatment the proportion of infected districts that are low has risen from 14.9% in 2010 to 87.1% in 2014 indicating that the large majority of the country is now classified as low infection. The Infection levels in districts followed-up have more than halved from 19.8% to 8.4 and the proportion of heavy or heavy/medium infections the prevalence before and after treatment has also fallen substantially (less than 4%). The programme implemented MDAs and surveys in some conflict areas and even targeted marginalized groups. In addition, it has been successful in keeping the gender balance among females and males SAC by the MDAs, and have achieved a considerable coverage among enrolled and non-enrolled SAC.

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IDENTIFICATION AND BIOLOGICAL CHARACTERIZATION OF NOVEL PHARMACOLOGICALLY ACTIVE COMPOUNDS OBTAINED FROM HIGH THROUGHPUT SCREENING OF *LEISHMANIA* PARASITE

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Leishmania is a trypanosomatid protozoan parasite which causes the disease leishmaniasis. The mode of transmission of this disease is via the bite of a sandfly, genus *Phlebotomus* (old world) and *Lutzomyia* (new world). Leishmaniasis is endemic in 88 countries worldwide and each day new cases emerge with increased morbidity and mortality. Presently, 12 million people are infected and around 350 million people are constantly at risk of acquiring this disease. The life cycle of *Leishmania* parasite exists between the sand fly (promastigote form) and the mammalian host (amastigote form). According to clinical manifestations leishmaniasis can be characterized as cutaneous, muco-cutaneous or visceral leishmaniasis; the latter being fatal. The disease itself is treatable but faces many challenges mainly due to emerging resistance and increased toxicity from current drugs. The therapeutic efficacy varies depending upon the disease pattern, species and geographical distribution of the parasite. An automated assay suitable for high throughput screening (HTS) is desperately needed which is cost effective and robust for the selection of a hit molecules for optimization as a therapeutic candidate. In an aim to identify compounds with activity against *L. donovani* DD8 parasites, a primary screen of 5000 structurally diverse compounds was performed using the promastigote viability assay (extracellular form) and an intracellular amastigote assay. Confirmation of activity was performed together with cytotoxicity studies against THP-1 (host cell) and HEK-293 cell lines. The HTS approach employed here resulted in the discovery of a new anti-leishmanial compound with an IC_{50} of 0.592 ± 0.139 μM against the intracellular form of the parasite and IC_{50} of 2.374 ± 0.859 μM against the extracellular form. Screening data and results from additional assays such as cidal-static and time to kill assays, plus % infectivity in the host cells after pre-incubation with the compound will be presented.

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VALIDATION OF POINT-OF-CARE MOLECULAR TESTING FOR DIAGNOSIS OF ULCERS DUE TO LEISHMANIA, FUNGI AND MYCOBACTERIA

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Due to the highly toxic nature of standard drugs used in the treatment of cutaneous leishmaniasis (CL), and overlapping clinical features of CL with ulcers due to fungal and mycobacterial infections, confirmatory diagnostic testing must be undertaken. We evaluated the performance characteristics of a handheld battery operated device for differentiation of *Leishmania* from known fungal and mycobacterial causes of cutaneous ulcers. Using ATCC strains of *Leishmania (L. V. braziliensis, L. V. panamensis, L. V. guyanensis*), mycobacteria (M. abscessus complex) and fungi (*Paracoccidioides brasiliensis*), we validated PalmPCR for detection of Leishmania, fungal, and mycobacterial species known to cause cutaneous ulcers. We further validated the device for detection of *Leishmania* from clinical specimens including filter paper lesion impressions, cytology brushes, and tissue. Respective primers targeted a conserved region of kinetoplast DNA (kDNA) for detection of Leishmania, pan-mycobacterial Hsp65, and pan-fungal D1D2 regions. PCR products were visualized using the EGel Go reader, a portable battery-operated system for agarose electrophoresis. Outcome measures were sensitivity and specificity, where conventional end-point or real time PCR was the reference standard. Compared to the reference standard, the PalmPCR device detected 100% of ATCC strains of Leishmania, fungi, and mycobacteria. There was no cross-reactivity of primers with any negative control. The PalmPCR device accurately categorized clinical specimens as positive or negative for *Leishmania* 94.1% of the time (16/17 specimens), yielding sensitivity and specificity of 91.7% and 90.0%, respectively. Positive predictive value and negative predictive value were 91.7% and 90.0%, respectively, for detection of Leishmania. We have verified that the PalmPCR device performs comparably to conventional end-point or real time PCR for the detection of Leishmania, fungi, and mycobacteria. This work has implications for CL diagnostic process improvement, and has the potential to improve point-of-care diagnostic sensitivity compared to conventional tests such as smear.

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EVALUATION OF MACROPHAGE ACTIVATION MARKER NEOPTERIN AS A PHARMACODYNAMIC BIOMARKER IN VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is caused by the *Leishmania* parasite, which replicates within host macrophages, thereby increasing the overall macrophage biomass which decreases again with waning parasitic infection. The aim of this study was to evaluate neopterin - a macrophage

activation marker - as possible pharmacodynamic (PD) marker to monitor VL treatment response, for which recrudescence of parasites is a long-term event which is difficult to predict. Samples were collected from VL patients in Sudan and Kenya receiving 1) a combination therapy of 1 liposomal amphotericin B (L-AmB) infusion followed by 10 days of miltefosine (L-AmB+MIL, 48 patients) or 2) 28 days of miltefosine (MIL, 48 patients). Neopterin was quantified with an ELISA kit in 498 plasma samples collected on miltefosine treatment day 1, 7, 11 (L-AmB+MIL), 14 and 28 (MIL) and 1 and 6 months after treatment. Baseline neopterin levels were elevated in all VL patients with a mean±SD of 135±93.4 nmol/L, regressing during treatment to 45.4±40.0 nmol/L (L-AmB+MIL) and 34.0±29.4 nmol/L (MIL). Neopterin levels were stable during the first 7 treatment days for monotherapy patients (111±70.4 nmol/L to 109±56.4 nmol/L), while levels of combination therapy patients halved (160±107 nmol/L to 74.0±65.8 nmol/L). 19 patients received rescue treatment within 6 months after treatment. These relapsed patients showed a significantly higher fold-increase in neopterin levels within 1 month after treatment (2.17±0.91) than patients that cured (1.04±0.81, $p<0.001$, Mann-Whitney U test). In combination therapy, neopterin concentrations one day after L-AmB infusion were significantly higher for cured (173±112 nmol/L) than for relapsing patients (98.2±49.6 nmol/L, $p<0.01$, Mann-Whitney U test). In conclusion, neopterin dynamics differed between the two treatment arms. The, possibly prognostic, initial surge in neopterin levels in cured combination therapy patients could imply an instant immunomodulatory effect of L-AmB on the Th1 response. Another possible marker in predicting treatment failure in VL is the relative neopterin concentration increase within 1 month after treatment.

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CHARACTERIZATION OF NEW CHEMICAL SCAFFOLDS FOR THE TREATMENT OF HUMAN AFRICAN TRYPANOSOMIASIS

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Trypanosoma brucei rhodesiense and *T. b. gambiense* are the aetiological agents of Human African Trypanosomiasis (HAT), a neglected, parasitic disease prevalent in sub-Saharan Africa. The number of cases of HAT has declined by over 80% in the last 20 years due to the introduction of rigorous surveillance and treatment programs. However, to achieve the goal of eliminating HAT as a public health problem by 2020, new molecules are needed as currently available drugs have limited efficacy, poor safety profiles and protracted, impractical administration schedules. This abstract describes preliminary ADME and mechanism of action studies of a novel class of promising anti-trypanocidal compounds. A novel class of anti-trypanocidal compounds was previously identified through a HTS whole cell screening campaign against *T.b. brucei*. Analogues were evaluated against *T.b. brucei* and the human infective subspecies *T.b. gambiense* and *T.b. rhodesiense* to build basic structure activity relationships and aid prioritization of lead compounds. Lead compounds underwent physiochemical and metabolism assessment and development of resistant strains was initiated to gain insights into possible molecular targets. Following evaluation of over >30 structural analogues, 3 compounds were prioritized based on anti-trypanocidal activity and medicinal chemistry properties. The compounds exhibited IC_{50} values ranging from 0.32 to 4.83 µM against the human infective subspecies *T.b. rhodesiense* and *T.b. gambiense* . Preliminary metabolism studies in human and murine microsomes reveled extensive non-NADPH mediated degradation that will need to be addressed through chemical modification of the molecules. In conclusion, the compounds identified in this study are potent, selective trypanocidal agents. Preliminary mechanism of action studies are currently in progress to identify to the molecular target of the molecules to allow the development of more potent analogues with improved metabolic stability which can be progressed along the drug discovery pipeline for HAT.

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A RETROSPECTIVE REVIEW OF THE HOSPITAL FOR TROPICAL DISEASES LEISHMANIASIS CASES IN 2013-2015

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The Hospital of Tropical Diseases (HTD) at University College Hospital London (UCLH) is a national center for treatment of Leishmaniasis. Guidelines for the Treatment of Leishmaniasis in travelers differ worldwide with varying outcomes. We retrospectively reviewed 38 cases of Leishmaniasis treated at HTD during 2013-15. We collected data on demographics, travel exposure, causative species, lesion location and number, treatment regimens and outcomes. 25(66%) were male, median age was 33.5 years old, range (17-81 years). We describe the severity at presentation by lesion number, size and location. 17(45%) of cases were Old World *Leishmania* infections. We used *Leishmania* DNA PCR to identify species of which 21(55%) patients had infections with *Leishmania Vianna*, a subspecies of *L. braziliensis*. 10 (26%) were acquired in the Peruvian Amazon. Amastigotes were identified in 20 (61%) of PCR positive biopsies. 3(9%) presented mucocutaneous leishmaniasis (MCL), 2 of 3 were L.Donovani infections acquired in Spain with vocal cord lesions and without skin involvement. 4(11%) presented with Visceral Leishmaniasis and treated with Intravenous (IV) Ambisome. All Cutaneous Leshmaniasis (CL) cases are managed as outpatients. 21(64%) Received daily IV Sodium Stibogluconate (SSB), 17(81%) of these *L. Vianna* infections. Most patients reported side effects with a wide range in severity. 6(18%) received treatment with oral Miltefosine with no treatment failures. In patients receiving IV SSB 2 (9%) failed on first line treatment, one was subsequently treated with oral Miltefosine and one with intra-lesional SSB both successfully. Side effects included mild reversible transaminitis, reversible QT prolongation, mild anaemia and Leucopenia. The majority of CL presenting to the HTD is in young adults with L.Viannia from the Peruvian Amazon and Central America. Risk of progression to MCL is low; treatment is well tolerated with a low relapse rate in our setting.

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A LOOP-MEDIATED IS OTHERMAL AMPLIFICATION (LAMP) KIT FOR MOLECULAR DETECTION OF *TRYPANOSOMA CRUZI* DNA: A FEASIBILITY STUDY

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Loop-mediated isothermal amplification (LAMP) tests have been developed as molecular tests for neglected parasitic diseases such as leishmaniasis or sleeping sickness. A LAMP test for *Trypanosoma cruzi*, the etiological agent of Chagas Disease (ChD), would allow a rapid and reliable diagnosis, in particular in cases of acute and congenital ChD (CChD). We evaluated the performance a *Trypanosoma cruzi* LAMP kit using purified DNA, spiked blood and clinical specimens. Quantitative PCR (qPCR) was used as a reference. Different extraction methods for LAMP were also evaluated. The LAMP reaction was performed at 62.5°C for 45 min. Analytical sensitivity was measured in ten-fold dilutions of CL Brener (TcVI) and Silvio X10 (TcI) DNA. Analytical specificity was measured using ten-fold dilutions of different *Leishmania* species and *Trypanosoma rangeli* DNAs as well as non-infected human DNA. Seronegative blood in EDTA (EB) or heparin (HB) was spiked with ten-fold dilutions of CL Brener. EB spiked blood was also used as dried blood spot (DBS). Stored DNA from EB clinical samples was tested, including 4 Congenital ChD cases, 5 Chronic ChD cases with low parasite loads, 10 immunosuppressed ChD patients and 5 seronegative controls. DNA extraction was done with a commercial kit (EB, HB and DBS

samples) and using the boil & spin (B&S) method (HB samples only). The *T. cruzi* LAMP kit showed better analytical sensitivity than qPCR in purified DNA specimens, especially for TcI DNA. Analytical sensitivity was 10^{-2} and 10^{-1} par.eq/mL from spiked EB and HB extracted by columns, respectively, and 10^{-2} par.eq/mL from HB using B&S. The analytical sensitivity in DBS samples was 10^{-2} par.eq/mL. *T. cruzi* LAMP was positive in congenital and immunosuppressed ChD samples spanning from 4.8 to 3,684 par.eq/ml, in agreement with qPCR. Chronic ChD samples were only detectable by qPCR, with Ct values below the limit of quantification. The kit was specific for *T. cruzi* DNA and samples from seropositive patients. The preliminary results demonstrate the potential of using *T. cruzi* LAMP as a molecular test for CChD and Chagas reactivation.

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ANALOGS OF THE NATURAL PRODUCT CHAMUVARININ TARGET THE TRYPANOSOMATID FOF1-ATP SYNTHASE MITOCHONDRIAL COMPLEX V

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Current treatments for trypanosomatid diseases are outdated, increasingly ineffective and associated with severe adverse effects. We recently reported trypanocidal activity of a series of novel synthetic bis-tetrahydropyran 1,4-triazole (B-THP-T) analogs based on the framework of the natural product chamuvarinin, an acetogenin first synthesized by our groups. Acetogenins are potent inhibitors of the human mitochondrial Complex I, however our compounds show potent inhibitory activity against bloodstream form *Trypanosoma brucei*, in which Complex I is absent, thus chamuvarinin must target another protein in the trypanosomatids. The mode of action of our B-THP-T compounds is unknown so this work aimed to identify their target in *Leishmania* major and *T. cruzi*. We synthesized a series of B-THP-T analogs for use in photo-affinity labeling (PAL), to covalently tag our target for protein target identification *in vivo*. We subsequently identified the FoF1-ATP synthase (mitochondrial complex V) as a potential target of our compounds using pull-down experiments. Next we undertook a series of biological analyses to validate this pull-down. By labeling B-THP-T-tagged proteins with the Cy5.5 fluorophore we confirmed that the target is mitochondrial by fluorescence co-localization. Using a luciferase-based ATP quantitation assay we show that B-THP-T compounds inhibit cellular ATP production and that they ablate oxidative phosphorylation. Taken together, these data indicate that our B-THP-T compounds are trypanocidal through their mitochondrial targeting of the FoF1-ATP synthase. We are using genetic manipulation of *T. cruzi* and L. major FoF1 ATP synthase subunits to validate these findings. With the target of our bis-tetrahydropyran 1,4-triazoles identified as mitochondrial complex V a structure-based approach can be used to optimize inhibitor potency and specificity.

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MULTIPLEX BEAD ASSAY FOR DETECTION OF ANTIBODY RESPONSES AGAINST *TRYPANOSOMA CRUZI* USING NOVEL PENTAVALENT ANTIGENS TCF26 AND TCF43

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Serological assays for Chagas disease are important for case detection, screening of donated biological materials, and could also be used in serosurveillance. We evaluated two novel multivalent antigens TcF26 and TcF43 in a multiplex bead assay (MBA). Each of these recombinant antigens expresses 5 reactive epitopes, previously reported to increase the sensitivity and specificity in single-plex assays. Each pentavalent antigen was optimally coupled to a specific classification of Seromap polystyrene beads for use in MBA. Assay performance parameters were determined with a panel of 108 samples previouslly characterized by radioimmunoprecipitation assay (RIPA) (44 were RIPA +, 64 RIPA -) plus 24 samples from a non-endemic area, and results were analyzed by receiver operating characteristic (ROC). For TcF26, positive samples had a average reactivity of 14,307 fluorescent units (range 68-25,419) and negatives 98.5 units (7-1,157) respectivelly. The cut-off for positivity was 711.5, providing 98.9% specificity, 93.3% sensitivity, negative predicted value (NPV) of 96.6% and positive predicted value (PPV) of 97.3%. For TcF43, positive samples had a average of 20,272 units (range 2,670-28,542) while negatives had 1,434 units (29-10,955); a positive cut-off was established at 3,654 units and provided 89.8% specificity, 97% sensitivity, NPV of 98.75% and PPV of 80.85%. The combined analysis of these results, using TcF43 for first line screening followed by confirmation with TcF26 results, resulted in sensitivity of 92.5%, specificity of 98.9%, NPV of 96.8% and PPV of 97.4%. The incorporation of these antigens into MBA would allow parallel testing against multiple analytes for Chagas. Although preliminary, these results suggest the potential advantages of detecting antibodies against these antigens in MBA testing for Chagas disease.

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MINIMIZING THE COST OF CONGENITAL CHAGAS DISEASE IN THE UNITED STATES THROUGH MATERNAL SCREENING

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Chagas disease, caused by *Trypanosoma cruzi*, is transmitted by insect vectors, as well as through blood transfusion, organ transplant, consumption of insect feces in food and water, and from mother to child during gestation. Programs of vector control and screening of blood products and transplant organs have been successful in reducing transmission. Congenital infection, however, could perpetuate Chagas indefinitely, even in countries with no or almost no autochthonous vector transmission. Even mothers who themselves have been infected congenitally and who are not symptomatic can transmit to their babies. About 30% of infected persons will develop lifelong cardiac or digestive complications that can be fatal. Treatment of infants with benznidazole has close to 100% cure rate, and efficacy in adults is estimated between 40% and 70%. This is the first study of the costs of screening and treatment for Chagas in the United States. We construct a decision-analytic model to find the cost-minimizing option, comparing the costs of testing and treatment, as indicated, for mothers and infants with the lifetime societal costs of no testing and consequent morbidity and mortality due to lack of treatment or late treatment. We find that a protocol of screening and treatment is cost-minimizing for all rates of congenital transmission between 1% and 10% and all levels of maternal prevalence above 0.2%. Lifetime societal savings due to screening and treatment are more than $4000 per Hispanic birth. There are more than 900,000 births to Hispanic women in the United States per year. Educating obstetricians to offer prenatal or cord blood serologic screening to Hispanic mothers makes it possible to treat mothers, infants, siblings, and other family members at risk of serious Chagas morbidity.

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SODIUM STIBOGLUCONATE AND PAROMOMYCIN FOR TREATING VISCERAL LEISHMANIASIS UNDER ROUTINE CONDITIONS IN EASTERN SUDAN

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objectives Among patients with primary and relapse visceral leishmaniasis (VL) in eastern Sudan, we determined the proportion eligible for treatment with sodium stibogluconate and paromomycin (SSG/PM) and, of these,

their demographic and clinical characteristics; initial treatment outcomes including adverse side effects requiring treatment discontinuation; treatment outcomes by 6 months; and risk factors associated with initial (slow responders) and late treatment failure (relapses and postkala- azar dermal leishmaniasis, PKDL). methods A retrospective cohort study in Tabarak Allah Hospital, Gedaref Province, eastern Sudan, from July 2011 to January 2014. results Of 1252 individuals diagnosed with VL (1151 primary and 101 relapses), 65% were eligible for SSG/PM including 83% children, almost half of them malnourished and anaemic. About 4% of individuals discontinued treatment due to side effects; 0.7% died during treatment. Initial cure was achieved in 93% of 774 primary cases and 77% of 35 relapse cases (P < 0.001). Among the 809 patients eligible for SSG/PM, 218 (27%) were lost to follow-up. Outcomes by six months among the 591 patients with available follow-up data were: definitive cure (n = 506; 86%), relapse (n = 38; 6%), treatment discontinuation (n = 33; 6%), PKDL (n = 7; 1%) and death (n = 7; 1%). Among those completing a full course of SSG/PM, relapses and under-fives were at significantly higher risk of early and late treatment failure, respectively. conclusion Whether SSG/PM as a first-line regimen is an undeniable progress compared to SSG monotherapy, it excluded a considerable proportion of VL patients due to drug safety concerns. We call for accelerated development of new drugs and treatment regimens to improve VL treatment in Sudan.

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CLINICAL EVALUATION OF A RAPID DIAGNOSTIC TEST FOR GAMBIENSE HUMAN AFRICAN TRYPANOSOMIASIS DEVELOPED USING RECOMBINANT ANTIGENS

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Diagnosis and treatment are the cornerstones of strategies to control *Trypanosoma brucei gambiense* human African trypanosomiasis (HAT). Screening using serological tests is the entry point in diagnostic algorithms. Until recently, the Card Agglutination Test for Trypanosomiasis (CATT) was the only screening tool used routinely. This test has a number of limitations, including bulk packaging and need for equipment and electricity. These have recently been addressed by the introduction of rapid diagnostic tests (RDTs). However, current RDTs are manufactured using native antigens that are costly and challenging to produce. An RDT developed using recombinant antigens was evaluated by passive screening in 10 sites, and by active screening in 5 mobile teams in the Democratic Republic of the Congo. CATT, an RDT produced using native antigens (SD BIOLINE HAT), and the new RDT (SD BIOLINE HAT 2.0) were used to screen 57,632 individuals and interpreted blindly by two readers. 260 HAT cases were confirmed by parasitology. When results of both active and passive screening were combined, the sensitivity of the screening tests was 62.5%, 59.0% and 71.2%, and the specificity was 99.2%, 98.9% and 98.1%, respectively. Sensitivity estimates were lower than previously reported, as some HAT cases were detected by one test and not the others. Sensitivity in passive screening (74.6%, 70.0% and 90.1%) was higher than in active (51.8%, 49.2% and 54.8%). The difference may be attributed to differential expression of antigens by parasites over time, resulting in immune responses to multiple antigens with advancing disease. While sensitivity of the tests was already high in passive screening, combining the SD BIOLINE HAT with the SD BIOLINE HAT 2.0 resulted in higher sensitivity (98.4%). This effect was more pronounced in active screening, where the sensitivity of all 3 tests was low, and combining the two RDTs resulted in a greatly improved sensitivity (83.0%). While the cost-effectiveness of algorithms including several screening tests should be investigated, this study has demonstrated that using two or more tests to screen for HAT greatly improves sensitivity.

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IMPROVED ACCESS TO DIAGNOSTICS FOR RHODESIENSE SLEEPING SICKNESS AROUND A CONSERVATION AREA IN MALAWI RESULTS IN EARLIER CASE DETECTION AND REDUCED MORTALITY

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Trypanosoma brucei rhodesiense human African trypanosomiasis (HAT) presents as an acute form of disease that develops rapidly, advancing into a neurological form that can only be treated with melarsoprol, an arsenic drug that has been reported to cause death to 5-10% of treated patients. It is a zoonosis that is transmitted by tsetse flies from wild and domestic animals, which are the main reservoirs. Elimination of rhodesiense HAT is challenging, particularly when the reservoirs are in conservation areas. Bringing diagnostic services for *rhodesiense* HAT closer to the populations that are at risk would increase chances of detecting cases in early stages of disease, when treatment is safer and more effective. Malawi is endemic for *rhodesiense* HAT, especially among populations living around conservation areas. Since 2010, between 18 and 35 new HAT cases have been reported annually in the country. Most of the infections occur around Vwaza Marsh Game Reserve, located in the north of Malawi. Until 2013, diagnosis of HAT in the region was only available at the Rumphi District Hospital, more than 60 km away from the game reserve. In 2013, the Ministry of Health of Malawi, in a partnership with FIND, initiated a project that radically enhanced passive detection of HAT in health facilities located around Vwaza Marsh. The capacity of 5 facilities to confirm the disease in clinical suspects was strengthened by upgrading laboratories and training technicians. Facilities were also supplied with equipment for parasitological diagnosis of *rhodesiense* HAT, including centrifuges and LED fluorescence microscopes. One facility was upgraded to perform LAMP, a highly sensitive and field applicable molecular test for detecting parasite DNA. Between August 2014 and March 2016, 49 HAT cases were diagnosed with this new strategy. Between January and March 2016, all of the 13 cases that were diagnosed were treated successfully. Compared to years before the project was initiated, data obtained so far indicate that the availability of diagnostic services closer to where people get infected promotes earlier case detection, better prognosis and reduced mortality.

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IMPROVED ACCESS TO PASSIVE SCREENING FOR GAMBIENSE HUMAN AFRICAN TRYPANOSOMIASIS DETECTS MOST PATIENTS IN FIRST STAGE DISEASE

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Majority of *gambiense* human African trypanosomiasis (HAT) cases detected in a new passive screening strategy implemented in Kongo Central province, Democratic Republic of the Congo, are in early or 1st stage disease, which is easier and safer to treat than late or 2nd stage disease. The strategy, which is integrated in the primary healthcare system, involves performing a HAT rapid diagnostic test (RDT) on a patient with symptoms suggestive of HAT but negative for malaria (or positive for malaria but still symptomatic after malaria treatment). If the HAT RDT is positive, the patient is referred for parasitological testing, and if confirmed, is treated. When a patient is negative by microscopy, further testing is done using a molecular test for parasite DNA known as LAMP. If a patient is at a facility that does not have LAMP, a blood sample is dried on filter paper and taken to a LAMP facility by a project motorcycle. LAMP positive patients are considered strong HAT suspects and undergo further

tests by microscopy, a requirement for case confirmation according to WHO guidelines. Roll-out of the strategy was preceded by introduction of HAT RDTs in all 597 public health facilities in the endemic region; 23 strategically located facilities were upgraded to perform confirmatory testing, including LED fluorescence microscopy, and 5 among them to also perform LAMP, with appropriate training in all facilities. This reduced the median distance travelled by referred patients to 11.2km, and the median distance that samples are transported for LAMP to 33km. 32 HAT cases were detected from July 2015 to March 2016; 19 (59%) of them were identified as suspects after testing positive with HAT RDTs at facilities that were not previously screening for HAT. Furthermore, 21 (66%) were in 1st stage disease, a significant paradigm shift from the past when most cases identified passively would be in late or 2nd stage disease. With the new strategy, the population at risk is fully covered, patients are screened for HAT on their first contact with the health system, and access to confirmatory diagnosis is improved. This is an important approach in the push to eliminate *gambiense* HAT.

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DIRECT MEMBRANE FEEDING AS SAYS FOR ESTABLISHING XENODIAGNOSIS STUDY IN VISCERAL LEISHMANIASIS: A PROOF OF CONCEPT STUDY

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The role of reservoir in transmission of anthroponotic VL is inadequately understood, and needs to be quickly elucidated in the context of an elimination program. With this view, a xenodiagnosis study to investigate the role of *Leishmania donovani*-infected individuals from across the infection spectrum in driving the transmission of endemic VL is need to be established. Further, to gain an insight into the mechanism that governs *Leishmania* infection, it is important to understand the context of the dose of parasite which is likely to affect the evolution of the disease. In the present study, we performed chick skin membrane feeding using sand fly to find out infective dose requisite for detection of the parasite in the sand fly midgut. colony of *Phlebotomus argentipes* developed using at Kala Azar Medical Research Center (KAMRC), Muzaffarpur, Bihar (India). Females of *P. argentipes* were fed on heat inactivated human blood for 1-2hr through a chick-skin membrane feeder containing 2×10^3, 2×10^4, 2.5×10^4, 5×10^4 and 2×10^5 promastigotes/0.5ml. A group of 20-30 unfed flies kept in feeding cup and the membrane feeders were placed upon the feeding cups. Membrane feeders were fixed in a circulating water bath maintained at 40^0C. Blood fed females were separated and kept in one pint paper cup provided with 30% sugar solution in incubator. Midguts of fully blood fed females were dissected at 24, 48, 72, and 96 hours post-infection and observed under microscope for the presence *Leishmania* promastigotes. The dose at which parasite detected was 2×10^5 promastigotes/0.5ml among several concentration used. Although the parasite detection was very less at 24 hr (1or 2) but at 48 and 72hr many parasite were observed. Further, no parasite was seen after 96 hr. Different promastigote forms were seen in the infected flies. Among these included procyclic promastigotes, nectomonads and haptomonads, metacyclic promastigotes. In conclusion, with this experiment, it can be concluded that sand flies colony developed is permissive for *Leishmania* parasite intake from human blood and outfitted for xenodiagnostic experiments.

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EFFECT OF N6-(FERROCENMETHYL)QUINAZOLIN-2,4,6-TRIAMINE ON MURINE MODEL OF CUTANEOUS LEISHMANIASIS

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Treatment for leishmaniasis began more than 100 years ago with the introduction of antimonials. Efforts have been done to improve efficacy, increase tolerability, diminish toxicity and parasite resistance as well as abate the cost. However, we are still far from reaching even half of those goals, meaning an urgent need to increase efforts in the search for compounds with improved characteristics. Recently, the synthesis, chemical characteristics, IC50 (50% inhibitory growth concentration) and *in vitro* parasite effects were described for N6-(ferrocenmethyl)quinazolin-2,4,6-triamine (H2). Here, we are reporting H2 effect over *Leishmania* mexicana infected mice on foot pad. BALB/c infected mice with MNYC/BZ/62/M379 *Leishmania* strain were treated IM with 4 mg kg-1 daily during 28 days in groups of five animals per cage. All mice had visible and measurable lesions at the beginning of the compound administration. One group for vehicle (20% DMSO) and other for meglumine antimoniate 120 mg kg-1 were used for comparison, with four groups of treatment for compound. Mice receiving vehicle or meglumine antimoniate shown the expected lesion growing, while mice receiving H2 compound stop the lesion growth during treatment, showing 50% less lesion size than vehicle or meglumine antimoniate in a model of resistant *Leishmania* mexicana infection. Any death was registered during this scheme of treatment, with dynamic animals. Lesion growth resumed two weeks after treatment ended, reaching 25% less lesion size than vehicle at third week after treatment end, while meglumine antimoniate was 10% up of the vehicle lesion size. This is our fifth experiment, with similar results. As a conclusion, N6-(ferrocenmethyl)quinazolin-2,4,6-triamine (H2) had better effect than meglumine antimoniate over lesion size by *Leishmania mexicana* infection. A complete parasite elimination has not been reached yet, however dose and treatment length could be increased in the future.

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ANTI-*LEISHMANIA* ANTIBODIES IN SAMPLES OF BLOOD DONORS FROM ENDEMIC AREAS OF BRAZIL

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In Brazil, in 2014, the incidence of visceral leishmaniasis (VL) was 3,453 cases. In endemic areas (EA), around 95% of VL individuals are asymptomatic and likely be undetected and accepted as blood donors. We assessed the prevalence of anti-*L. infantum*-rK39 antibodies among blood donors from Brazilian EA. IgG antibodies were surveyed by ELISA using recombinant K39 antigen provided by Infectious Disease Research Institute, USA (ELISA-rK39). The study was carried out with 6,125 blood donors from 13 Brazilian states. Reactivity index (RI) was calculated for each sample and the median of RI≥1.0 were determined for each state. Samples yielding RI≥1.0 in ELISA-rK39 were tested in ELISA using *Leishmania major*-like antigen (ELISA-*L. major*), using K28 recombinant antigen (ELISA-rK28), DAT, indirect fluorescent immunoassay (IFI) and Kalazar rapid diagnostic test (RDT) (Inbios). Anti-rK39-IgG antibodies were detected in 322/6,125 samples (5.2%). RI≥1.0 varied from 1.003 to 10.770 (median = 1.470). Anti-rK28-IgG antibodies were detected in 28/322 samples (8.7%). RI≥1.0

varied from 1.013 to 9.546 (median = 1.732). For DAT were considered positive titres > 1,600, and 3 samples were positive (0.9%). In ELISA-*L. major*, 141 samples were positive for (43.8%); in IFI, 11 (3.4%) and in RDT, 17 (5.3%). From these results, it seems that rK39 is more sensitive to detect asymptomatic infections. Anyhow, the great prevalence of IgG antibodies achieved in blood samples from asymptomatic donors points out to the risk of transfusional leishmaniasis in endemic areas. As in those areas, it is not easy to differentiate transfusion- or vector-mediated transmission; the occurrence of transmission by transfusion is probably underestimated and raises concerns on blood transfusion safety.

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DETECTION AND QUANTITATION OF *LEISHMANIA* DNA BY REAL TIME PCR IN WHOLE BLOOD AND SKIN LESION BIOPSY OF ETHIOPIAN CUTANEOUS LEISHMANIASIS (ECL) PATIENTS PRESENTING WITH VARIED SKIN LESION TYPES

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Parasitological diagnosis of Old World Cutaneous Leishmaniasis (CL) in skin lesion specimens remains to be insensitive. This study was conducted to explore if PCR-based diagnosis of Ethiopian Cutaneous Leishmaniasis (ECL) could be a feasible option. The study aimed to detect *Leishmania* DNA in skin lesions and peripheral blood specimens of ECL patients using real time PCR, and to determine the sensitivity in patients presenting with localized CL (LCL) and mucocutaneous leishmaniasis (MCL). Patients were those referred to Leishmaniasis Research and Diagnostic Laboratory (LRDL) at Addis Ababa University. 48 patients with diagnosis of LCL (n=27) and MCL (n=21) were included. NNN cultures and smears were made alongside PCR using dermal scraps, cotton swabs, PBMC, whole blood, and buffy coat specimens. Primers 13A and 13B were used to amplify a region of 120bp mini-circle DNA sequences. Nodular and ulcerative lesions were commonest in LCL; while in MCL single ulcers involving mucosa and accompanying inflamed and edematous tissue were common. 70.4% of LCL and 71.4% of MCL patients were positive parasitologically. Using kDNA real time PCR, 74.1% of LCL and 71.4% of MCL patients were positive in skin biopsies; whereas in cotton swab samples, positivity rates by real time PCR were 81.5% and 90.5% in LCL and MCL patients respectively. The sensitivity of kDNA real time PCR using biopsy specimen from LCL patients varied from 74.1% to 89.5% cf. rates of 71.4 - 75.0% in MCL. Skin biopsies cf. skin slit specimens gave higher yields of parasitological diagnosis by NNN medium. The later gave higher yield by microscopy. Swabs of skin slit specimens gave higher PCR positive rates cf. biopsied material. PBMC or buffy coat specimens gave a negative parasitology or kDNA real time PCR. These results indicate that cotton swab specimens obtained from skin slit of lesions are preferred samples for the diagnosis of ECL using real time PCR. Inability to diagnose ECL from buffy coat and PBMC by PCR and parasitological procedures has major implications about diagnosis and transmission. Our data indicate that sand flies have to feed directly on the lesions for transmission to take place.

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SEROPREVALENCE AND RISK FACTORS OF BRUCELLOSIS IN SMALL RUMINANTS SLAUGHTERED AT DEBRE ZIET AND MODJO EXPORT ABATTOIRS, ETHIOPIA

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Brucellosis is a global zoonotic disease and major public and animal health problem in many parts of the world, particularly in places where livestock is a major source of food and income. This cross-sectional study was conducted between November 2012 and May 2013 to determine the seroprevalence and assess potential risk factors of brucellosis in small ruminants in five export abattoirs at Debre Ziet and Modjo, Oromia Regional State, Ethiopia. Serology and questionnaire were the methods used. In this investigation, 853 sera samples of 485 caprines and 368 ovines brought for slaughter were selected randomly. The Rose Bengal plate test and complement fixation test were conducted using sera samples at National Animal Health Diagnostic and Investigation Center (NAHDIC) serology laboratory. Data collection sheets were used to gather information on possible risk factors believed to influence the occurrence of Brucella infection in small ruminants such as age, species, breed, body condition score, and origin of small ruminants. Brucellosis was found in 17 (1.99%) and 15 (1.76%) small ruminants using the Rose Bengal plate test and complement fixation test, respectively. The univariate and multivariate logistic regression analysis showed that age and body condition score of the animals were risk factors to Brucella infection ($p = 0.008$ and $p = 0.001$, respectively) in small ruminants. In conclusion, based on this survey, brucellosis is a potential problem in small ruminants in Ethiopia that should be further explored.

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INFLUENCE OF CO-INFECTION ON CARRIAGE OF HUMAN PATHOGENS IN BROWN RATS FROM AN URBAN SLUM SETTING

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Rapid urbanization in developing countries has led to the spread of slum settlements and high infestation with the brown rat, *Rattus norvegicus*, which harbours multiple pathogenic and commensal agents. Yet we know little about the prevalence of pathogens in this reservoir and how co-infection influences transmission of these agents and the risk of spillover infection to humans. We conducted a survey of brown rats in 2014 in a slum community in the city of Salvador (Bahia-Brazil) to elaborate an interaction network between rat-borne infectious agents. We sampled rat urine and kidney imprints and feces to identify/quantify Leptospira interrogans and helminth species. We performed generalized linear models to identify significant effects of coinfection on the presence and intensity of each helminth species and L. interrogans controlling for environmental factors and the demographics and body condition of rats. Final models were combined to build the interaction network. We trapped a total of 299 rats of which 71% and 39% were carriers of the human pathogens *Leptospira interrogans* and the nematode *Angiostrongylus cantonensis*, respectively. We identified 10 other helminths among rats; the most prevalent were *Strongyloides* sp. (97%) and *Nippostrongylus brasiliensis* (41%), of which the latter was present at higher intensity with higher intensities of both Strongyloides sp. and *L. interrogans*. The intensity of *A. cantonensis* was also higher when rats had higher intensities of *Strongyloides* sp.. However no influence of *L. interrogans* was found. Moreover, the prevalence of *A. cantonensis* was negatively associated with co-parasitism with *N. brasiliensis* and *Heterakis spumosa.* Our findings confirm that rats carry zoonotic pathogens, such as *A. cantonensis* (the causal agent of eosinophilic meningitis in humans), which are under-recognized and expanding causes of human disease in urban slum settings. Furthermore, coinfection with parasites may significantly modify carriage of human pathogens in rats and contribute to variation in the transmission risk between regions.

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FIELD OBSERVATIONAL STUDY EVALUATING THE SPILLOVER OF ANTIBIOTIC-RESISTANT BACTERIA BETWEEN DIFFERENT VARIETIES OF CHICKENS AND RURAL IN NORTHERN ECUADOR

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The twentieth century industrialization of animal husbandry has resulted in dramatic increases in animal production. It has also led to departures from traditional agricultural techniques including the pervasive and widespread administration of antimicrobial agents. Antibiotics are administered in animal production facilities for both prophylactic and therapeutic purposes. Over-exposure of growth promotion antibiotics has resulted in the worldwide emergence of antibiotic-resistant bacterial strains in most species of livestock and poultry. Such strains are increasingly spreading from large animal production facilities into human populations, which presents a pressing public health concern. Both physicians and veterinarians are faced with a declining supply of effective antibiotics. In Ecuador, small scale farming operations in rural communities often prescribes high amounts of antibiotics for poultry raised for meat consumption known as "production chickens" (e.g. broilers). In contrast, free-ranging local varieties of household chickens receive almost no antibiotics and are important as potential bridge hosts introducing antibiotic-resistant strains from production chickens to humans. Through a cross-sectional village-scale approach, we analyze the relationships between antibiotic-resistant prevalence between household and production chickens along with a subset of humans living near household chickens. For each household, we evaluate the prevalence of antibiotic-resistant *Escherichia coli* in household chickens and the spatial relationship to the nearest production chicken coop. This investigation is relevant to many other tropical countries where development agencies commonly introduce production chickens as means of supporting micro-development. There is a pressing concern to better understand how antibiotic-resistant bacteria spread from conventional to non-conventional animal breeds and these impacts on surrounding human populations.

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EXAMINING RATS IN THE MARSHALL ISLANDS AS A POSSIBLE ANIMAL RESERVOIR FOR *MYCOBACTERIUM TUBERCULOSIS* AND ATYPICAL MYCOBACTERIA

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The Marshall Islands have some of the highest rates in the world for both Mycobacterium tuberculosis and Atypical mycobacteria infection. The Marshall Islands and specifically Majuro General Hospital, in the capital, have significant problems with rat infestations and rodent control. To investigate the possibility of an animal reservoir for these diverse groups of Mycobacteria a total of ten rats from two areas in the hospital were captured in live traps. Five rats were captured in and around the nurses station of the intensive care unit. The other five rats were captured in the storage area near the surgical ward. More rats could have been captured, however hospital staff sometimes turned the rats loose or tripped the traps closed during the night. All of the captured rats were drowned in fresh water the the surgical pathology department, washed and then autopsied for evidence of granulomas and lymphadenopathy. The heart and lungs were then harvested and saved in formalin containers. Histologic sections of the heart and lungs showed no evidence of granulomas and acid fast staining was negative for mycobacterial organisms. Three of the rats appeared well nourished, but ill. None of the rats had lymphadenopathy or grossly evident liver disease. However, nine out of ten of the rats showed evidence of chronic lymphocytic bronchitis in a pattern that would be significant in human patients. A control group of two rats was trapped. One from the mountains of New Mexico and one rat from the area of China town near the medical school. Both rats were processed in the same manner. These rats showed no evidence of bronchitis, although the rat from New Mexico did have several foci of coccidioidomycosis. This is only intended to be a preliminary study. It was not possible to culture the lungs for infectious agents and the urine and feces of the rats was not examined during this study. Some hospital staff were concerned that other animal reservoirs might be present such as feral dogs, cats and domesticated pigs. Further studies might include wild dogs or local pigs as well as larger numbers of rats analyzed with more sensitive technology such and the Genexpert and lung cultures.

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MODELING ENVIRONMENTAL TRANSMISSION OF ZOONOSIS IN MULTISPECIES SYSTEMS

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Animals harbor important pathogens that impact both livestock and human health. In addition, the increased use of transmission models to study animal disease has highlighted the need to consider the host-pathogen interaction in the context of environmental and ecosystem drivers of infection transmission. We developed a novel use of disease transmission models to study the environmental transmission of zoonotic disease in multispecies herding systems characteristic of many pastoral economies in the emerging world. Specifically, we examine the role of diversity on pathogen persistence demonstrating that, depending on environmental factors and the mechanisms of host species interaction, diversity can have differing effects on the transmission dynamics. Our results extend prior work of the role of diversity in pathogen persistence by providing a mechanistic understanding of how environmental processes can impact modes of transmission. These models will not only help inform public health interventions but could be crucial for conservation decisions in areas characterized by livestock-wildlife interphases.

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RISK FACTORS FOR ACUTE HUMAN BRUCELLOSIS IN NORTHERN TANZANIA

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Brucellosis is an important zoonotic cause of febrile illness with chronic sequelae, but little is known about transmission pathways in sub-Saharan Africa hampering the design of control programs. We conducted a prospective cohort study of acute human brucellosis in northern Tanzania. We enrolled pediatric and adult patients with fever from two referral

hospitals in Moshi, Tanzania. We administered a standardized risk factor questionnaire and performed *Brucella* microagglutination testing on acute and convalescent serum. Cases were patients who had either a four-fold rise in titer between paired serum samples or a single reciprocal titer ≥160. Controls had titers <20 in both serum samples. We calculated odds ratios (OR) for individual behaviors and combined behaviors to form exposure scales to livestock and livestock body fluids. Of 1,446 patients enrolled from February 2012 through September 2014, we identified 50 (3.5%) acute brucellosis cases and 512 controls. Remaining participants either had titers >20 - <160 or supplied only acute serum. Median (interquartile range) age of cases was 31 (23, 40) years, 33 (66.0%) of 50 were female, and 20 (43.5%) of 46 were from rural areas. On bivariate analysis, increasing age was associated with brucellosis (OR 1.1 per year, p<0.01). Birthing goats (OR 8.8, p=0.01) or livestock (OR 6.2, p=0.02); consuming raw livestock blood (OR 2.7, p=0.03); exposure to goat (OR 4.0, p<0.01) or pig blood (OR 3.7, p<0.01); feeding cattle (OR 3.9, p<0.01), goats (OR 2.8, p<0.01), or pigs (OR 7.0, p<0.01); and cleaning waste of cattle (OR 4.1, p<0.01) or pigs (OR 5.6, p<0.01) were associated with brucellosis. Brucellosis was associated with high levels of contact with livestock measured by the exposure scales (OR 3.2, p<0.05). No association was found between raw milk consumption and acute disease. We found that livestock contact was an important risk factor for brucellosis in northern Tanzania, and that risk varied both by type of livestock contact and by livestock species. Many of the risk factors identified are behaviors potentially modifiable by targeted interventions.

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INFLUENZA A VIRUS AMONG PIG AND DUCK POPULATIONS IN RURAL BACKYARDS IN GUATEMALA, 2013-2014

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The human-animal interface may favor the emergence of novel influenza A virus (IAV) strains that might pose a threat to human and animal health. We conducted monthly cross-sectional surveys of backyard pigs and ducks from November 2013-October 2014 on the Pacific coast of Guatemala where IAV was isolated from migratory waterfowl. We collected nasal swabs (pigs), tracheal and cloacal swabs (ducks), and blood from both species. Households with each of the species were selected randomly based on an expected IAV prevalence of 10%. One of every two pigs and 1/7 ducks were selected by convenience within each household; animals could be re-tested in different months. We tested swabs for IAV RNA by rRT-PCR targeting the universal IAV matrix gene, and subtyped the matrix positive samples by rRT-PCR targeting the 2009 pandemic N1 gene. We tested sera to detect exposure to IAV using a commercial ELISA kit, and subtyped ELISA positive sera from ducks by HI against a H5N3 low pathogenic strain isolated from waterfowl. An average of 32 households with pigs and 46 households with ducks were sampled monthly. We collected a total of 669 samples from pigs and 1090 samples from ducks. We detected 7/669 (1%; CI 95%: 0.4-2) rRT-PCR positive pig samples; none tested positive for pandemic N1. Only 1/1090 (0.1%; CI 95%: 0.1-0.5) of tracheal swabs from ducks tested positive by rRT-PCR (cloacal results pending). We detected antibodies against IAV in 85/669 (13%; CI 95%: 10-16) of sera from pigs and 98/1090 (9%; CI 95%:7-11) from ducks. Among seropositive ducks, 1/98 (1%; CI 95%: 1-6) were reactive to the H5N3 virus. HI results may suggest exposure of ducks to IAV from wild waterfowl, but isolation and sequencing of IAV strains from positive swab samples to identify IAV viruses circulating in pig and duck populations is pending. We found low percentages of current IAV infections in these backyard animals. Serologic results are limited by potential re-testing of individuals and knowing the animal age, but suggest low exposure to IAV. Interspecies transmission of IAV and the implementation of an integrated surveillance of IAV at this setting, where multiple hosts interact, should be evaluated.

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LEPTOSPIRA SEROPREVALENCE AND RISK FACTORS IN HEALTH CENTER PATIENTS IN HOIMA DISTRICT, WESTERN UGANDA

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The burden of human leptospirosis in Uganda is unknown, but is suspected to comprise a significant portion of undifferentiated febrile illnesses. The study objectives were to estimate the prevalence of *Leptospira* antibodies and risk factors for seropositivity in humans visiting two health centers in Hoima District, Uganda. 359 patients were recruited at the Kikuube and Kigorobya Health Center IV's during March and April 2014. Every non-pregnant adult over the age of 18 presenting to the health center, either as a patient or as a caregiver, was eligible. Interviews were conducted by clinical officers and a blood sample was taken by lab technicians. Exposure variables included demographics, exposure to animals, past medical history and domestic environment. Sera were tested by the Microscopic Agglutination Test (MAT) using eight Leptospira serovars from different serogroups. 126 study participants (35.0%, 95% CI 30.2-40.3%) were seropositive (MAT titer >= 100) against any serovar. The highest prevalence (19.8%, 95% CI 15.9-24.4%) was against *L. borgpetersenii* sv Nigeria (serogroup Pyrogenes) with 71 seropositive cases. The prevalence of probable leptospirosis (MAT titer >=800) was 1.9% (95% CI 0.9-4.2%) and was uniquely related to serovar Nigeria. Probable leptospirosis was statistically significantly associated with self-reported malaria in the past year (p=0.048). The few participants who reported having skinned cattle in the two weeks prior to their blood sample (n=6) had a relative risk of 2.6 (p=0.036) for seropositivity against any serovar. This is to our knowledge the first study of the prevalence of antibodies to *Leptospira* serovars in humans in Uganda. The seropositivity to leptospirosis and the specific seroprevalence of 20% against L. borgpetersenii sv Nigeria suggests high exposure in this population. Individuals participating in cattle skinning may also be at higher risk for exposure. Overall, leptospirosis may represent an underappreciated burden of illness in Hoima, Uganda.

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IMPACT OF REV1 LIVESTOCK VACCINATION ON THE RISK OF HUMAN BRUCELLOSIS IN AZERBAIJAN

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Brucellosis is one the most common and widely spread zoonotic diseases in the world. Effective control of the disease is, in general, focused on reducing or eliminating brucellosis in livestock through vaccination. However, despite the availability of effective control measures, brucellosis continues to pose a public health risk in both developed and developing countries. The objective of our study was to evaluate the impact of Rev1 small ruminant brucellosis vaccination on the risk of human brucellosis in Azerbaijan. We used monthly time-series data on human brucellosis cases in Azerbaijan before and after a Rev1 small ruminant vaccination campaign

(1995-2013). We used an interrupted time-series framework to estimate the relative and absolute indirect effect of the livestock vaccination policy on human risk. Incident risk was calucated for age groups before and after vaccination. To identify spatial variations in temporal reporting trends, we used the scan statistic. Additionally, we mapped temporal trajectories of human brucellosis incidence, by administrative district, using a spatially lagged incidence rate in a segmented regression model. Our results showed a post vaccination decline in human brucellosis of ~1% per month. Overall, human brucellosis was reduced by 34% by 2013. Risk reduction was greatest in the 20-29 age group. Despite national declines in human brucellosis, we identified spatial changes in the case distribution characterized by a geographic expansion and an increasing incidence among districts clustered in the south-east, compared to a decrease elsewhere in the country. In conclusion, our findings support the human health benefits of livestock vaccination. However, we found spatial variation in the impact of the vaccination program that can be used to target future efforts. Our findings highlight the use of integrating spatial and quasi-experimental techniques to evaluate the progress of public health interventions.

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CLIMATE CHANGE AND VECTOR-BORNE DISEASES: USE OF PARTICIPATORY EPIDEMIOLOGY TO INVESTIGATE EXPERIENCES IN VULNERABLE, CATTLE-KEEPING HOUSEHOLDS IN TANZANIA

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Climate change is predicted to increase incidence of vector-borne diseases (VBDs) in humans, however little is known about the impact on animal VBDs in vulnerable areas where livestock are a primary livelihood strategy. In the absence of historical data with which to examine the inter-relation between climate and disease, participatory epidemiological (PE) methods were used with Maasai pastoralists of Monduli district, Northern Tanzania to establish local observations on two major VBDs of cattle, namely East Coast Fever (ECF) and Animal Trypanasomiasis (AT). Data were collected between November 2014 and January 2015 in ten randomly selected villages of arid and semi-arid lands involving gender segregated groups (10 men groups and 9 women groups). Matrix scoring for both men and women groups confirmed that Maasai easily recognise these VBDs. ECF and AT ranked amongst the top five most important cattle diseases in the district with strong agreement between informant groups (Kendall's W = 0.399 for men and 0.451 for women; $p < 0.01$). All groups associated ECF with the wet season or directly after rainy season while AT was more variable throughout the year, with more cases reported in dry seasons. Likewise, different villages reported seasonal differences in occurrence of disease vectors (*Rhipicephalus appendiculutus* and Tsetse flies). Comparing 2014 to 1984, participant groups consistently reported declines in rainfall, vegetation cover and quality pasture, as well as increases in severe drought. Experiences with ECF/AT and vector abundance between these time periods was more variable across villages, and likely relates to changes in climate and animal management practices over the last 30 years. This baseline study is the first to document the inter-relation between climate and cattle diseases from the pastoralist perspective. Preliminary analyses reveal a complex interplay between human, animal and environmental factors, understanding of which is urgently required to devise approaches to mitigate effects of climate change in vulnerable areas.

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ONE HEALTH WORLDWIDE: EMERGING INFECTIOUS DISEASES, GLOBAL HEALTH VETERINARIANS, AND ZOONOTIC SURVEILLANCE

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What do zoos have to do with Global Health? How are veterinarians engaging in pandemic prevention? Do non-zoonotic veterinary pathogens impact human health? Given that most zoonotic pathogens are from wildlife, surveillance of pathogens in wildlife is garnering more attention from domestic and international health organizations. The National Zoological Park's Smithsonian Global Health Program (SGHP) is a team of wildlife veterinarians, veterinary pathologists, biologists, physicians, and other public health professionals tasked with investigating and combatting emerging diseases worldwide. One Health ventures that SGHP is invested in include: human-animal interface surveillance (through USAID|PREDICT), xenosurveillance for MERS coronavirus and Zika virus, and emerging ulcerative dermatitides in African megafauna. SGHP engages in international collaboration and capacity building of diagnostic and public health infrastructure in developing tropical countries. Veterinarians form an integral part of this corps of experts, and their unique and often overlooked understanding of the interactions between humans and wildlife make them an essential presence on the front lines of emerging zoonoses detection and prevention. Although much has been devoted to understanding the human consequences of well-known zoonotic outbreaks, such as the Ebola virus, very little focus has been placed on poorly studied zoonoses or non-zoonotic wildlife diseases and the potential risk they present to human health. This has led to the emergence of serious threats from previously "benign" diseases such as Zika virus. There is even evidence that non-zoonotic animal pathogens may play a role in the severity and transmissibility of zoonotic diseases, as when the competency of certain arbovirus vectors increases as a result of co-infection with animal-borne filariasis. It is the mission of multidisciplinary One Health groups such as SGHP to tackle these challenges from all angles and combat pandemic threats. Collaboration with other health professionals is a vital component of ongoing integrated strategies in improving health outcomes globally.

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SEROPREVALENCE AND THE RISK FACTORS ASSOCIATED WITH TOXOPLASMOSIS IN WOMEN RECEIVED ANTENATAL CONSULTATION (ANC) AND DOMESTIC CARNIVORES IN DAKAR

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Toxoplasmosis is a cosmopolitan zoonosis caused by *Toxoplasma gondii* manifested by fetal loss mainly in sheep and humans. The identified definitive host is the cat while all domestic mammals such as wild birds and humans are intermediate hosts. Disease sporadic pace, its modes of transmission are manifold. The object of this study was to evaluate the seroprevalence of toxoplasmosis and the risk factors in pregnant women and domestic carnivores in Dakar. For this, blood samples of 100 pregnant women, 141 cats and 120 dogs were collected and sera were analyzed. Regarding the acquisition of risk factors, a questionnaire was associated with each sample. Two agglutination series tests showed that the women surveyed were infested at 50 ± 9.8% for toxoplasmosis. Multivariate analysis showed that 43% of these women have had an abortion and of those, 53% were positive for toxoplasmosis. After analysis of risk factors, professional status and milk consumption predispose women to be contaminated ($p < 0.05$ and odds ratio> 1) with toxoplasmosis. For carnivores, the agglutination test shows that the infestation is higher for

definitive hosts (cats 55.37 ± 9%) compared to dogs (43.97 ± 8%). These high prevalence confirms that this parasite is prevalent in an endemic form in the region especially with the close cohabitation between women and cats. They must motivate the strengthening and systematization of screening tests during pregnancy.

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UPDATE Q FEVER STATUS FROM THE RUMINANT PLACENTAS, THAILAND 2014-2015

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Q fever, caused by *Coxiella burnetii* is a zoonotic disease, found worldwide. The ruminants such as cows, buffalo, goats, and sheep are reservoirs. The most common clinical sign in livestock is abortion, but infected adult animals almost never show clinical signs. The main transmission route is by inhalation in humans, and possibly animals as well. As ruminant placenta is occasionally sold in wet markets in Thailand for human consumption, at-risk people include the farmers, veterinarians, animal husbandry workers, placenta-merchants, and cooks, and potentially the general public. Human Q Fever cases were reported in Thailand. We aimed to know the Q fever status in ruminants in Thailand. Approximately 300 samples of the cotyledon part of ruminant placenta were collected by convenience sampling from each region. The samples were grossly normal, though some were retained placentas. The samples were extracted for DNA and tested with the real-time polymerase chain reaction (PCR) technique, which targets the IS1111. The tests were run in the Thai governmental veterinary laboratory system, comprised of the National Institute of Animal Health (the central region), and seven Veterinary Research and Development Centers (regional labs). The positive percentage of results, by region in Thailand was 63.84 (203/318) in the east, 50.9 (170/334) in the upper north, 45.62 (151/331) in the west, 38.82 (125/322) in the central, 24.77 (81/327) in the lower north-east, 19.73 (59/299) in the upper north-east, 8.46 (28/331) in the lower north, and 7.51 (16/213) in the south region. The total percentage for the whole country was 32.46 (833/2475). Q fever occurred in ruminants in Thailand without showing abortions, as occasionally seen in other countries. Good sanitation such as disinfection, wearing personal protective equipment, including gloves, masks, and boots, especially during the parturition time, and routinely cleaning the animal house should be done by the farmers, and veterinarians. The placenta-merchants and cooks should wear gloves when handling raw placenta and thoroughly cook placenta if served for human consumption.

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DOCUMENTING THE SEROPREVALENCE OF ZOONOTIC AND VECTOR BORNE DISEASES IN RURAL NICARAGUA

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Rural communities, particularly in low and middle income nations, are at high risk of zoonotic and vector borne diseases. The rural poor in these parts of the world are disproportionately affected, but the extent of which is largely undescribed, in part due to limitations on surveillance capacity in resource constrained settings. In Nicaragua, the tropical climate is particularly conducive to transmission of mosquito borne viruses, such as Dengue, Chikungunya, and Zika, and frequent close contact with domestic and other animals in rural communities facilitates transmission of zoonotic pathogens. In order to understand the extent to which infections like these are circulating within the region, we undertook a cross-sectional study to estimate the seroprevalence of infectious diseases among agricultural workers in the Pacific lowlands of Nicaragua. Workers of a large sugar estate underwent routine, annual occupational health screenings from October 2015-June 2016. Age and sex of workers were available. From June-October 2015, blood was also collected from domestic animals in the same region. Serum from humans and animals were available for testing and were subjected to ELISA assay for detection of antibodies to various pathogens (ELISA for IgM or IgG and and MAT for *Leptospira* reactivity). Workers were mostly male (89%) and young (median age 29yrs). In humans, we detected a high prevalence of antibodies to *Leptospira* (33%) and also detected antibodies to hantavirus (6%) and *Trypanosoma cruzi* (0.5%). Dogs had evidence of leptospirosis (12%) and *T. cruzi* (5%). Testing for seroprevalence of other infections, including Dengue, Chikungunya, West Nile, and Zika are ongoing. Our data suggests that zoonotic and vector borne pathogens are likely established and endemic, provides evidence that hantavirus may be a threat, and documents the presence of Chagas disease in this region of Nicaragua. This new data can inform public health and veterinary practices and suggest areas for targeted surveillance and intervention strategies.

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SURVEILLANCE OF AMOEBIC KERATITIS-CAUSING *ACANTHAMOEBAE* FOR BACTERIAL ENDOSYMBIONTS

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Acanthamoebae are causative pathogens of several infections including amoebic keratitis (AK), a potentially blinding eye infection. Acanthamoebae isolated from the environment and from corneas of patients with AK are known to harbor bacterial endosymbionts belonging to chlamydiales, rickettsiales, or legionellales. Acanthamoebae harboring Chlamydia-like endosymbionts have demonstrated enhanced production of cytopathic effect on fibroblast monolayers, suggesting that endosymbionts may increase virulence. We sought to illuminate the potential bacterial endosymbionts present in clinical isolates of acanthamoebae identified at our reference parasitology laboratory. Isolates of *Acanthamoeba* spp. obtained from our biobank of surplus, anonymized, corneal scrapings from 2012-2015 were screened for endosymbionts by PCR. Isolated DNA was amplified in PCR reactions with 3 separate primer pairs,detecting bacteria belonging to orders chlamydiales, rickettsiales, or legionellales. 3 primer pairs specific to the 18s rRNA gene of *Acanthamoeba* spp. were used for amplification of *Acanthamoeba* DNA. Sanger sequencing of PCR products was performed, followed by BLAST analysis for sequence homology and species identification. We screened

27 clinical isolates of *Acanthamoeba* spp. for organisms known to act as endosymbionts as described above. Five strains of *Acanthamoeba* (19%) from corneal scrapings were found to contain bacterial DNA belonging to legionellales (2 *A. polyphaga* (40%), others not speciated). 3 of the clinical isolates (11%) contained members of the rickettsiales (2 *A.castellani* (66%), 1 A. palestinensis (33%)). One strain (4%, not speciated) contained a member of the chlamydiales, and sequencing revealed this to be *Neochlamydia hartmannellae*. The remaining isolates of *Acanthamoeba* may contain other endosymbionts undetectable by our assays. Organisms known to act as bacterial endosymbionts are prevalent in corneal scrapings containing AK-causing *Acanthamoeba*. Whether these potential endosymbionts contribute to virulence is unknown, but offers a potential avenue for investigation of novel therapeutics in AK.

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OPTIMIZED METHODS OF GENOMIC DNA EXTRACTION AND MYCOPLASMA DECONTAMINATION FROM DIFFERENT SPECIES OF PATHOGENIC AMOEBAE

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Free-living amoebae (FLA) are generally regarded as innocuous soil organisms; however, a handful of species are capable of causing human disease. *Naegleria fowleri*, *Acanthamoeba* spp. and *Balamuthia mandrillaris* are recognized as etiologic agents of amoebic encephalitis. Acanthamoeba are also responsible for the sight-threatening infection amoebic keratitis, particularly among contact lens wearers. A rate-limiting step in genomic analyses of pathogenic FLA is DNA extraction. Many commercially available DNA isolation kits are useful for PCR but fail to provide high yields of pure genomic DNA for molecular studies. We examined different in-house methods for the capacity to extract FLA DNA. We found that an optimized phenol-chloroform-based purification method was applicable to different species of amoebae when compared to a commercial kit. While similar to other standard methods of extraction, this modified procedure resulted in DNA of high yield and quality as it likely takes into consideration the high amounts of carbohydrates and nucleases found in amoebic cells. Mycoplasma contamination is also of great concern in cultures of pathogenic amoebae, especially in strains propagated in mammalian cell lines. We examined different methods of decontamination of Balamuthia cultures harboring mycoplasma that were resistant to commonly used antibiotics. The methods were based on the rapid treatment of amoebic cysts with non-amoebicidal levels of detergents and/or acids to eliminate the contaminating bacteria as determined by PCR. Our non-antibiotic-based approach was effective in curing cultures of Balamuthia from mycoplasma without affecting viability of the amoebae. Taken together, the use of reliable methods of DNA extraction combined with simple and economic procedures of bacterial decontamination offers an effective approach to produce genomic DNA from pathogenic amoebae on a large scale for a range of molecular genetic analyses.

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DIAGNOSTIC ACCURACY OF AMEBIC COLITIS BY COLONOSCOPY

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Amebiasis, caused by *Entamoeba histolytica*, is rapidly increasing as a sexually transmitted infection in Japan. It has been also reported that asymptomatic amoebic colitis incidentally diagnosed by colonoscopy recently increased. Although etiologic diagnosis of intestinal ulcer mostly relies on histological examination of biopsy specimen, it is still unclear whether the method has enough sensitivity to rule out amoebic colitis. We collected samples from suspected cases of amoebic colitis by colonoscopy. Microscopic examination, culture and PCR of aspirated fluid, as well as histological examination of biopsy specimen, were performed. Diagnosis of amoebic colitis was made when either test was positive. Of the 23 suspected cases, amoebic colitis was diagnosed in 13 cases (57%). The proportions of co-infection with HIV, HBV, HCV, and syphilis were 54%, 54%, 8% and 39%, respectively. At the colonoscopy, 10 patients (77%) had clinical symptoms, including diarrhea (62%), abdominal pain (23%), bloody stool (15%), fever (15%), and nausea/vomiting (15%), whereas 3 (23%) were completely asymptomatic. One case accompanied amoebic liver abscess. The sensitivities of microscopy, culture, PCR and histopathology were 69%, 23%, 67% and 46%, respectively. The proportion of cases with abdominal symptom was statistically higher in histological negative than that in histological positive (p 0.04). Three clinical isolates, including 2 isolates from asymptomatic cases, were stably passaged as a xenic culture. Either diagnostic tool including histological examination used in this study doesn't have enough sensitivity (23-69%) for the diagnosis of amoebic colitis by colonoscopy, indicating that combination of multiple methods be needed for the accurate diagnosis. Also, we established 3 clinical isolates from aspirated fluids of the lesions. We will perform genetic and phenotypic analyses of these clinical isolates in order to elucidate the pathogenesis of amoebic colitis, especially asymptomatic chronic infection.

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GUT MICROBIOME CHANGE PRIOR TO THE ONSET OF AMEBIASIS

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Amebiasis is one of the causes of severe diarrhea in young infants <5 years of age in low and middle income countries. In our previous work we observed that the composition of the microbiota was markedly different in ≤ 2 year old children experiencing amebic diarrhea compared to those asymptomatically colonized. In particular, a high level of the pathobiont *Prevotella copri* was associated with symptomatic amebiasis. We postulated that either gut dysbiosis and the domination of the microbiota by *P. copri* increased the probability that an *Entamoeba histolytica* infection would be symptomatic or the resistance of *P. copri* to host-derived reactive oxygen allowed it expand after the amebic parasite caused gut inflammation and diarrhea. To distinguish between these two possibilities, we examined the 16S microbiome profile in 16 surveillance specimens collected and stored in the study biobank 89 ± 77 days prior to the occurrence of *E. histolytica* positive diarrhea. In our preliminary analysis we have observed a stronger *P. copri* signal in the samples preceding disease. This seems to indicate that a preexisting *P. copri* expansion dispose children to symptomatic disease and the previous result was not a consequence of *E. histolytica* infection. We also observed that even although *P. copri* was higher in diarrheal cases than the amount detected in *E. histolytica* positive surveillance samples the onset of diarrhea actually led to an expansion of the *Enterobacteria* at the expense of both *P. copri* and *Bifidobacteriales* species which dominate the pre- diarrhea microbiome in these children. The Shannon diversity index was as expected lower in pre-diarrheal and diarrheal samples compared to samples from 2-year old controls p<0.05. Additional studies are planned to determine the significance of these results.

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GENOMIC AND TRANSCRIPTOMIC COMPARISON OF CLINICAL AND NON-CLINICAL STRAINS OF "BRAIN-EATING" AMEBA *NAEGLERIA FOWLERI*

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The "brain-eating" free-living ameba, *Naegleria fowleri*, causes rare, but severe brain infection, known as primary amebic meningoencephalitis (PAM) that is almost always fatal. The ameba is found globally in warm fresh waters, hot springs, and waterparks. Recently, *N. fowleri* has been found to colonize public tap water systems in US linking the death of a young child. This coincides with an observed geographical spread in PAM cases to the colder Northern States. A major issue affecting our ability to investigate and prevent PAM infections is the ubiquitous nature of this pathogen. It is unclear why PAM cases are so rare while people are likely to be routinely exposed to *N. fowleri*. Variability in the ameba virulence may explain this. However, a genotyping system capable of categorizing *N. fowleri* strains based on their virulence does not exist. The existing genotyping categorizes the US strains of *N. fowleri* into only three broad genotypes. Thus, this genotyping is also not ideal for linking exposures with cases. Here, we performed Illumina Hiseq to do whole genome sequencing of 38 *N. fowleri* strains (30 from clinical cases and 8 from environmental samples) representing all three genotypes. We also performed RNAseq in a subset of *N. fowleri* strains to identify gene expression differences between the clinical and environmental strains. The *de novo* assembly of mitochondrial genome shows that it is ~49.5 KB in size. Preliminary analysis identifies SNPs in the mitochondrial genomes, some of which are genotype-specific and some are unique ("private") to a particular strain. The private SNPs can be utilized to develop a more discriminatory genotyping system, which will allow source tracking in clinical cases, and conduct molecular epidemiological surveys. The *de novo* assembly of nuclear genome of *N. fowleri* shows that it is ~27.5 MB in size, and contained in <600 contigs with a G-C content of ~34%. The only reference genome of *N. fowleri* available publicly is dispersed in >1100 contigs suggesting a superiority of our assembly. Analysis of nuclear genome and transcriptomic data is currently ongoing. Key findings will be presented in the ASTMH Meeting.

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IMPACT OF NOVEL *ENTAMOEBA* SPECIES ON DIARRHEAL INFECTIONS IN SOUTH AFRICA

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South Africa has been significantly impacted by diarrheal infections. The purpose of our study is to identify the etiology, including novel species and burden of parasitic diarrheal disease in South Africa. We collected diarrheal and non- diarrheal stool samples from the rural and urban communities of South Africa. DNA was extracted and a diagnostic taqman qPCR assay capable of identifying protozoan parasites (*Entamoeba histolytica*, *Cryptosporidium* and *Giardia*) was performed. In both locations approximately half of the samples were qPCR positive: 105 (49%) from rural areas; *Cryptosporidium* (24%) *E. histolytica* (3%) and *Giardia* (22%) and 113 (42%) from the urban areas; *Cryptosporidium* (11%), *E. histolytica* (9%) and *Giardia* (22%). We further identified *Entamoeba* species using a new assay with increased sensitivity. Our main finding was the presence of *E. bangladeshi* in eight different stool samples. This was an interesting finding as *E. bangladeshi* has not been previously reported outside Bangladesh. Consistent with previous findings, *E. moshkovskii* was not found in these populations and as predicted *E. histolytica* and, non-pathogenic *E. dispar* positive samples were also identified. Novel *Entamoeba spp* have been identified in different endemic regions of the world but have not been studied in S. African populations. We are currently performing additional analysis on the unspeciated *Entamoeba* positive samples as a part of a systematic approach to identify novel clinically relevant species. Our preliminary results suggest that novel *Entamoeba* species may potentially exist in the South African population.

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WHOLE GENOME SEQUENCING OF *CYCLOSPORA CAYETANENSIS* OOCYSTS PURIFIED FROM HUMAN STOOL SAMPLES

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Cyclospora cayetanensis is a coccidian parasite that causes cyclosporiasis, an intestinal infection characterized by acute diarrhea in humans. Since the 1990s, CDC and local public health departments have conducted numerous investigations of cyclosporiasis outbreaks. These investigations are currently hampered by the lack of molecular epidemiological tools. In order to improve outbreak response, we aim to develop a subtyping method that would allow linkage of cases to clusters and to implicated food sources. Attempts to propagate *Cyclospora in vitro* and *in vivo* have so far been unsuccessful, so stool samples from infected humans are the only available source for this parasite. Here, we describe the optimization of methods to purify *Cyclospora* oocysts from stool, extract genomic DNA and prepare next generation sequencing libraries from ultra-low quantities of DNA. Using these methods, genomic sequences from 10 geographically distinct *C. cayetanensis* samples were obtained by Illumina sequencing. The genome is approximately 44.5 Mbp with a GC content of 51.9%. Preliminary genomic comparisons indicated an overall low intra-species genomic variation. More detailed sequence comparisons are now in progress to develop SNP profiling of annotated regions of the genomes for epidemiological purposes, and to identify subtyping markers that can assist in future outbreak investigations. A public Bioproject database in NCBI is being created to host whole genome assemblies as part of this work.

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GENOME-WIDE SEARCH TO IDENTIFY IMMUNODOMINANT *BABESIA MICROTI* ANTIGENS FOR DIAGNOSTICS AND VACCINATION

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Babesiosis, caused by intraerythrocytic protozoan of the genus Babesia is transmitted by Ixodid ticks but also through blood transfusion. The highest prevalence of both tick and transfusion-transmitted infection occurs in the United States where *Babesia microti* infection is endemic in the Northeast and upper Midwest. Clinical manifestations range from asymptomatic infection to fulminant disease that could be fatal. There is no FDA-licensed vaccine to ameliorate parasite burden and clinical disease and no laboratory test for diagnosis of acute infection or for screening of blood donors. In spite of the recent availability of the full genome sequence of *B. microti*, there is a scarcity of well-defined, immunodominant *B. microti* antigens for development of diagnostic assays for blood donor screening or for vaccine efficacy studies. By applying a combination of genomics data mining and screening of whole-genome-fragment-phage-display libraries expressing the open reading frames of *B. microti* on immune sera from *B. microti* infected patients, we have identified over 50 immuno-dominant antigens of unknown biological function. Twenty-four of the highest reactivity *B. microti* antigens have been produced as recombinant protein in *E. coli*. Of these, 12 antigens displayed a strong EIA reactivity with sera

from *B. microti* infected individuals from endemic areas in Connecticut suggesting their potential as diagnostic antigens. Bioinformatics analyses and studies on their biochemical and cellular characterization as well as their value as diagnostic vaccine antigens are in progress.

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GAMMA IRRADIATED SOLUBLE EXTRACTS OF *TOXOPLASMA GONDII* TACHYZOITES INDUCED BETTER HUMORAL AND CELLULAR IMMUNE RESPONSE DUE TO PREFERENTIAL UPTAKE BY APCS SCAVENGER RECEPTORS

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Toxoplasmosis occurs in one-third of the adult world population, without adequate vaccines and causing disease in fetus or specific groups. Aside to sterilizing effect, gamma radiation acts on antigens inducing enhanced antisera production against snake venoms or cell and humoral response to recombinant leprosy proteins. Gamma radiation affects proteins directly or indirectly in water by action of oxidant radicals from water radiolysis. Early reports showed gamma irradiated crotoxin had enhanced uptake by macrophages, limited by scavenger receptors competitors, as probucol. Irradiated tachyzoites induced adequate immune response with protection, attributed to mitotic death and DNA damage. Irradiated proteins could take a part in this process and we study the immune response induced by gamma irradiated soluble extracts of *Toxoplasma gondii* tachyzoites, using mice immunized with native proteins as controls. Mice immunized with irradiated extracts without adjuvants showed significant protection after challenge with ME-49 ($p<0.05$) and RH ($p<0.0001$) strains compared to controls. There are increased specific and high avidity IgG production ($p<0.05$) when compare d to controls group. By flow cytometry and *in vitro* culture, spleens of mice immunized irradiated extract presented increased proliferation of CD4+, CD8+ and B cells and IFN-γ production as compared to controls. J774 cells had increased uptake of biotinylated irradiated extracts as compared to the uptake of native extract ($p<0.05$), due to longer and continuous uptake. All these data points to an alternative and effective uptake and immune processing of irradiated *T. gondii* extracts, probably due to specific receptor of oxidized proteins as scavenger receptors, resulting in enhanced immunity. This data also implies that irradiated proteins could be involved in the protection induced by irradiated parasites. Use of antigen gamma radiation can be a simple process to enhance vaccine efficiency, avoiding the use of adjuvants.

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PHYLOGENETIC ANALYSIS OF *BLASTOCYSTIS* SPP. ISOLATES IN CLINICAL STOOL SAMPLES FROM BRAZIL

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Blastocystis spp. is an organism described as enteroparasite protozoan, commonly found in stool samples from humans. Several subtypes have been described in humans, but pathogenic potential and aspects epidemiological are still controversial. The aim of the present study was to investigate *Blastocystis* subtypes (STs) from patients of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC/FMUSP), Brazil. *Blastocystis* spp. positive stool samples diagnosed in Section of Parasitology of Central Laboratory (HC-FMUSP) were used for DNA isolation. Polymerase chain reaction (PCR) was performed using specific primers targeting the small subunit of rRNA gene. Direct DNA sequencing of PCR products was performed, and the DNA sequences were aligned and compared to other sequences present in GenBank database. Phylogenetic analysis was inferred using the Neighbor-Joining method by MEGA6 software. Additionally, *Blastocystis* STs were identified by determining the exact match or closest similarity against all known *Blastocystis* STs using www.pubmlst.org/blastocystis. Four STs were identified: ST1 (16.0%), ST2 (8.0%), ST3 (68.0%) and ST6 (8.0%). Allele nos. 34 and 36 were the most frequent haplotypes. The present study is one of the few that generates STs data from human population in Brazil, confirming the absence of ST4. Another important finding is the presence of ST6, rarely detected in human isolates. Subtype prevalence involving human samples may contribute to the monitoring of infection transmission of *Blastocystis* spp in endemic areas, and in future, explain any pathogenic aspects related to distinct subtypes.

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DISTRIBUTION AND HUMAN-INFECTIVE POTENTIAL OF *CRYPTOSPORIDIUM*, *GIARDIA DUODENALIS* AND *ENTEROCYTOZOON BIENEUSI* GENOTYPES IN STORM OVERFLOW AND WASTEWATER IN SHANGHAI, CHINA

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Few data are available on the distributions of *Cryptosporidium*, *Giardia duodenalis*, and *Enterocytozoon bieneusi* genotypes and subtypes in storm overflow from urban areas. In the present study, 40 overflow samples were collected from two pump stations during July-September in 2012 and 2014 in Shanghai, China, with 40 raw wastewater samples from the same stations as controls. They were analyzed by using PCR for *Cryptosporidium* spp. (targeting the small subunit rRNA gene), *G. duodenalis* (targeting the triosphosphate isomerase, β-giardin, and glutamate dehydrogenase genes), and *E. bieneusi* (targeting the ribosomal internal transcribed spacer). Genotypes of these pathogens were identified by sequence analysis of PCR products. Samples that contained *C. hominis*, *C. parvum*, *C. viatorum*, *C. ubiquitum*, and *C. meleagridis* were further subtyped by sequence analyses of the 60-kDa glycoprotein gene. *C. hominis*, *C. parvum*, *C. ubiquitum* and *C. viatorum* were the dominant *Cryptosporidium* species. *C. baileyi*, *C. muris*, and *C. meleagridis* were also found in both wastewater and overflow samples. The *C. hominis* and *C. parvum* subtypes were common ones previously found in humans in China, but *C. ubiquitum* belonged to two novel subtype families and *C. viatorum* was found in China for the first time. There were eight Group 1 *E. bieneusi* genotypes in wastewater and storm overflow with genotype D as the dominant one. For *G. duodenalis*, subassemblage AII was the dominant genotype in these samples. There were no significant differences in the distribution of *Cryptosporidium* species and *E. bieneusi* and *G. duodenalis* genotypes between wastewater and overflow samples. These results reaffirm that storm overflow is potentially a significant contamination source of human pathogens in surface water and more attention should be paid to its roles in environmental transport of waterborne pathogens.

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DETECTION OF *CYCLOSPORA CAYETANENSIS* IN FOOD AND CLINICAL SAMPLES USING A GELIFIED REAL-TIME PCR AS SAY

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Cyclospora cayetanensis is a coccidian parasite associated with numerous foodborne outbreaks. Current diagnostic detection of *C. cayetanensis* relies on microscopy, albeit this technique does not identify the parasite to the species level. PCR methods in general are more complex than microscopy and most protocols require multiple steps to set up reactions. The gelified PCR technology allows the development of ready-to-use PCR assays that minimize labor and quality issues. By using a pre-mixed, pre-loaded and gelified reagents the execution of PCR tests is reduced to a few very simple steps; i.e., (i) addition of DNA template to pre-mixed, pre-aliquoted gelified reagents, (ii) mixing of content, and (iii) centrifugation and placement into the real-time PCR thermal cycler for execution of PCR run. We developed a streamlined real-time gelified PCR method to detect *C. cayetanensis* to the species level in foods and clinical samples. The assay was designed with an internal amplification quality control to monitor amplification, ensure adequate quality of the DNA preparations, and troubleshoot technical glitches. The evaluation of the method in food samples were performed with cilantro spiked with different concentrations of *C. cayetanensis* oocysts. Preliminary results indicated that this assay can detect approximately 10 oocysts of *C. cayetanensis* seeded in 25g of cilantro. The sensitivity and specificity of the technique was evaluated using a total of 38 human stool specimens, of which 34 were microscopically positive for *Cyclospora* sp, collected during outbreak investigations in the U.S. No false-positive or false negative results were obtained. The gelified assay was also evaluated regarding its stability at different temperatures, including room temperature, and 30°C. The results from these experiments revealed that the assay may be stable for up to 2 months when pre-loaded vessels with the gelified qPCR mix were stored at 30°C and 4 months at room temperature.

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AMIXICILE: A POTENTIAL ALTERNATIVE TREATMENT FOR TRICHOMONIASIS

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Trichomoniasis is a sexually transmitted infection in humans caused by *Trichomonas vaginalis*. This parasitic infection is the leading causative agent of vaginitis in women and urethritis in men worldwide. Currently, metronidazole is the most common drug therapy for this infection, but resistance is becoming increasingly prevalent. Amixicile is a novel inhibitor of pyruvate:ferrodoxin oxidoreductase in anaerobic parasites. This study compared the efficacy of a newly synthesized drug, amixicile, to that of both metronidazole and the synthetic precursor of amixicile, nitazoxanide, in inhibiting or killing *T. vaginalis in vitro*. One standard strain from ATCC and six unidentified patient-isolated strains of the *T. vaginalis* parasite were included in the study. The three drug treatments were compared, each at concentrations varying from 1.56 - 200µM. Under anaerobic conditions *in vitro*, the minimum inhibitory concentrations were determined for each of the three drugs against the seven *T. vaginalis* strains tested. The MIC for metronidazole, nitazoxanide, and amixicile were approximately 12.5µM, 100µM, and 6.25µM, respectively. To determine whether the inhibitory effect was reversible, antibiotic-exposed isolates were again placed in fresh culture media and incubated for 48 hours. Amixicile appeared to have the greatest efficacy in clearing the parasite at low drug concentrations. These sensitivity tests show amixicile's potential in serving as an alternative to metronidazole in the treatment of the *T. vaginalis* infection in humans. Further testing is required to confirm the effect of amixicile in more clinical isolates and *in vivo*.

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DRUG RESISTANCE IN *BABESIA* PARASITES THAT INFECT HUMANS

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In our previous work screening compounds against *Babesia in vitro*, we found that atovaquone was the most potent available agent against this parasite of growing significance in human health. We then studied atovaquone resistance in the parasite *in vitro* using continuous drug pressure on *B. divergens* cultures. In our mixed culture base pair changes resulted in M64V/I amino acid changes. These amino acid changes were associated with an increase in IC50 to atovaquone. Sequence analysis of the cytochrome b gene revealed that these mutations are likely in the Qo region and correlate with the M133V mutation described in *Plasmodium*. We then compared these *in vitro* results to those from patients with smear positivity for *B. microti* over several transmission seasons. In most samples we found a wild type cytochrome b gene. In one patient with documented chronic disease we identified a base pair change leading to an amino acid change adjacent to the conserved PEWY region, a Y202C amino acid change that correlated with the described Y268C mutation seen in *Plasmodium*. As the number of immunosuppressed patients that are at risk of *B. microti* infection increases, there is concern for worsening treatment failures. We propose that more formal studies of partner drugs and novel agents should be done given the current limited options to treat *B. microti* infections.

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A RAPID MOLECULAR TEST TO DIAGNOSE *TOXOPLASMA GONDII* IN MICE AND HUMAN SAMPLES

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Toxoplasmosis is a parasitic disease caused by *Toxoplasma gondii*. This infection is prevalent in humans and animals worldwide. It has been estimated that one-third of the world population has been exposed to this parasite. It is acquired by ingesting tissue cysts from undercooked or raw meat, consuming food or drink water contaminated with oocysts shed by felids, or by accidentally ingesting oocysts from the environment. Although the course of the primary infection is usually subclinical and the vast majority of infected human populations remain asymptomatic, the infection can cause significant morbidity and mortality in certain groups. The symptoms include encephalitis, chorioretinitis, congenital infection and neonatal mortality. Current diagnosis is based on detection of Toxoplasma-specific IgM and IgG. In this study, we established and optimized a diagnostic test using Recombinase Polymerase Amplification (RPA) assay for the molecular diagnosis of *T. gondii* in Lima. . The RPA assay does not require a thermocycler or other specialized equipment and can be adapted to lateral flow detection, so it may be performed on the field. For identification and amplification by RPA, we selected a gene fragment within a gene (B1) that was conserved across *T. gondii* strains. The limit of the RPA assays that we performed has a sensibility of detection

of 0.5 parasites (about 50 fg of DNA), while the conventional PCR detects about 1 parasite (100 fg of DNA). We also analyzed eight samples (all of them were given a positive diagnosis for Toxoplasmosis), five were positive by RPA and only three were positive by conventional PCR. The specificity was 100 % and did not detect other parasites like *Trypanosoma cruzi, Cryptosporidium, Cyclospora, Leishmania* and *Plasmodium*, Therefore, we observe that this assay can be applied to human samples for the diagnosis of the infection. The sensitivity of our methodology is 63% (5/8) and that of conventional PCR is 38% (3/8), The RPA assay can be further improved by combining it with lateral flow test. These results are very encouraging and suggest that RPA could be used as a novel molecular diagnosis technique for *T. gondii* infection.

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IMMUNOBLOT FOR DIFFERENTIATION BETWEEN ACUTE AND CHRONIC INFECTION OF *TOXOPLASMA GONDII* USING A MURINE MODEL

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Toxoplasmosis is caused by the obligate intracellular parasitic protozoan, *Toxoplasma gondii*. This infectious agent is generally foodborne and enters the host through ingestion of raw or undercooked meat derived from infected animals. Usually the acute toxoplasmosis occurs in immunosuppressed patients, while most of the people remain in the chronic phase without symptoms. Current diagnostic tests are not able to differentiate between acute and chronic infection. In this study we validated the response against *T. gondii* antigens in an experimental mouse model of Toxoplasmosis in two stages of infection. Five female Swiss Webster mice were infected orally with cyst of *T. gondii* ME49 strain and four mice were inoculated with saline solution (control group). Blood was collected 8, 15, 30 and 60 days post-infection. The mouse brain infections were confirmed by conventional PCR and by optical microscopy. The immunoblot was performed using the tachyzoite lysate proteins of RH strain as antigen, and IgM and IgG as detection antibodies. For the IgM immunoblot we identified three immunogenic proteins with low molecular weight (25, 30 and 39 kDa); these bands appeared within 8 and 15 days post infection and gradually disappeared until 60 days. For the IgG immunoblot we observed the same antigenic bands and also the specific recognition of a 28 kDa protein. The band corresponding to this protein was observed after 30 days (chronic stage) post-infection, and afterwards its intensity increased progressively. We can also detect other high molecular weight immunogenic proteins. The detection of these proteins by immunoblotting might be useful to estimate the stage and development of infection in the diagnosis of the toxoplasmosis disease.

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STANDARDIZATION OF AN ON-BEAD SANDWICH ELISA FOR THE DETECTION OF *TOXOPLASMA GONDII* ANTIGEN SAG1

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Toxoplasmosis is a disease caused by *Toxoplasma gondii*. Infected adults generally develop a chronic infection and remains asymptomatic. However, if a person becomes immunosuppressed, the infection can be reactivated and cause severe damage in different tissues, mainly leading to a diffuse encephalopathy. The diagnosis of this disease is principally done by the detection of antibodies in blood; nonetheless, IgG antibodies can persist live long in immunocompetent toxoplasma-infected individuals, making it difficult to differentiate between a recent and an older infection. On the other hand, the antibody production can be impaired in immunocompromised patients. For these reasons, it is important to design new diagnostic tests that can detect circulating antigens (CAg). Although some tests have been already developed, it is necessary to create more easy, simple and accurate approaches for the detection of CAg. The use of microparticles provides a large surface-to-volume ratio that facilitates detection, stability, and manipulation. The aim of this study is to standardize an on-bead sandwich ELISA for the detection of the main surface antigen of *T. gondii* (SAG1 or P30). In order to do this, we used magnetic microparticles Dynabeads-M270 Epoxy and got a good performance using a concentration of 0.05 mg. The standardization process was done using Total Lysate Antigen from parasite culture of the RH strain. The optical density (OD) of the positive control was 2.6 times the OD of the negative control and the minimum amount of SAG1 that could be detected was 100 ng (considering that it accounts for 3 to 5% of the total *T. gondii* protein). We processed 7 mice serum samples (4 were infected with RH strain and 3 with ME49 strain). The ODs of the samples from RH-infected mice were 4 to 7 times higher than the ODs of the negative control. However, the ODs of the serum samples from the ME49-infected mice were very similar to negative controls. The future direction of this project is to perform this assay using other types of microparticles and new commercially available antibodies in order to increase the sensibility and specificity in the toxoplasmosis diagnosis.

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URBAN PIGEONS (*COLUMBA LIVIA DOMESTICA*) AS SOURCE OF ENVIRONMENTAL SPREAD OF *CRYPTOSPORIDIUM* WITH ZOONOTIC POTENTIAL

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Urban pigeons, also known as domestic pigeons or Rock Pigeons (*Columba livia domestica*), are birds of Columbiformes order quite common in many urban centers, living in close contact with humans. Three hundred and eleven pigeons were captured with appropriate cages found at home and in human's peridomicile in the state of Rio de Janeiro, Brazil. The bottom of the cages was protected by a plastic, to facilitate the collection of feces; after the birds defecated they were released. Microscopic diagnosis was performed to verify the presence of *Cryptosporidium* oocysts using the technique of centrifugal flotation in sugar solution. In positive samples a DNA extraction was performed, followed by PCR technique for the 18S target gene. All samples obtained from the Nested-PCR reaction were stained and observed on agarose gel and subsequently purified. After this procedure they were sequenced at BLAST platform, and a search of sequences obtained was performed to determine their identities and possible similarities and homologies with previously deposited species in GenBank '. Phylogenetic analyzes were performed using the MEGA 6 software. Of a total of 387 fecal samples 16.31% (54/311) were positive in microscopy, and of these, 5,68% (22/387) were sequenced and identified two species *C. meleagridis* and *C. baylei*. Species *C. meleagridis* is worrying in terms of public health, because the pigeons are easily adapted to human environment, whether for the abundant supply of shelter, lack of predators, lots of food available; in addition, *C. meleagridis* is the third most prevalent species in humans.

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GENETIC DIVERSITY AND TRANSMISSION DYNAMICS OF *CRYPTOSPORIDIUM* PARASITES IN CATTLE OF SOUTHERN GHANA

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The coccidian parasite *Cryptosporidium* causes enteric disease in human beings, domestic animals and wild vertebrates. Infection is often self-limiting in immunocompetent individuals, but could be severe and chronic in persons with compromised immune system. Studies have shown a strong association between human cryptosporidiosis and contact with potentially infected cattle, particularly pre-weaned calves. Whereas studies on cryptosporidiosis in Ghana have focused on microscopy identification of the pathogen in immunocompromised people, limited information exists on the genetic composition of this zoonotic parasite species. The study reported in this paper investigated the genetic diversity of *Cryptosporidium* parasite across different age groups of cattle from the southern part of Ghana. Stool samples were collected from cattle and were processed by formol-ether concentration. After morphological identification of the oocysts, genomic DNA was extracted from the concentrate, followed by polymerase chain reaction assay to identify the species of the parasites. PCR positive samples were subjected to Restriction Fragment Length Polymorphism analysis using SspI for species diagnosis or VspI for genotyping of *C. parvum*. Out of the 90 stool samples analysed so far, 11 were positive for *C. parvum*. Further analysis are ongoing to ascertain if the strain is similar to those in human beings.

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PATHOGENICITY OF *DIENTAMOEBA FRAGILIS*

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Dientamoeba fragilis is a trichomonad protozoan which is commonly reported throughout the world. Despite its initial discovery over 100 years ago remarkably little is known about this parasite. The parasites life cycle and mode of transmission are poorly defined, and controversy surrounds the pathogenic potential of this organism. This talk will highlight the latest clinical studies, animal studies and the recent publication of the *D. fragilis* transcriptome from which several potential markers of pathogenicity were described. Molecular detection of the parasite will also be discussed.

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COMPARISON OF TWO RECOMBINANT ANTIGENS FOR DIAGNOSIS OF CHRONIC HUMAN FASCIOLIASIS

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Fascioliasis has been recognized as an emerging/reemerging zoonotic disease with an estimated prevalence of up to 17 million people infected and 180 million at risk for infection worldwide. In the United States, fascioliasis should be considered in immigrants, refugees or travelers with eosinophilia. Public health laboratories need a simple and reliable method for diagnosis of fascioliasis to identify and treat cases. The recognized laboratory test of choice for diagnosis of fascioliasis is detection of disease specific antibodies, most commonly using excretory-secretory antigens for detection of IgG antibodies. Recently, recombinant proteins such as FhSAP2 and Fh-CLP-1, have been used in an ELISA based format to detect IgG antibodies. To develop a better assay that could be used for diagnostic and surveillance, we used the GST-FhSAP2 recombinant antigen and Fh-CLP-1 to develop Western blot (WB) to detect *Fasciola hepatica* total IgG antibodies. We evaluated the assays using well-characterized sera from persons with or without fascioliasis. The sensitivity and specificity of GST-FhSAP2 and FhCLP1 WB were similar at 94% and 98% and 100% and 99%, respectively. For the multiplex immunoblot, the sensitivity and specificity were 100% and 98%. Although the defined positive sample size is small, the study supports the previous study results. In conclusion, the GST-FhSAP2 and FhCLP1 antigens perform well in immunoblot format separately or combined and can be readily adopted by public health and commercial reference laboratories for clinical diagnosis of *F. hepatica* infection in refugees, immigrants, and travelers with eosinophilia in the United States.

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APICAL SODIUM-DEPENDENT BILE ACID TRANSPORTER OF *CLONORCHIS SINENSIS*: 3D STRUCTURE AND FUNCTIONALITY

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Apical sodium-dependent bile acid transporter (ASBT, SLC10A2) plays a key role in the bile acid recycling. Bile acid uptake of ASBT is electrophysiologically coupled with co-transport of sodium ion. When *Clonorchis sinensis* survive in the bile duct, an extreme environment of bile juice, *C. sinensis* ASBT (CsASBT) could serve as an important contributor in its bile-taxis and survival. In this respect, we report here a homology modeling and molecular docking of CsASBT as a drug target. Its complete coding sequence was 1,641 bp long and encoded a polypeptide of 546 amino residues. Inward-facing (IF) and outward-facing (OF) conformations of CsASBT 3D model were generated by homology modeling using the crystal structures of *Neisseria meningitidis* (PDB ID: 3zuy) and *Yersinia frederiksenii* (PDB ID: 4n7x) as templates. The modeled structures were further refined and verified for higher reliability. IF- and OF-CsASBT were built in region of 185-492 aa and 189-489 aa, respectively, whereas remaining region was predicted to be disordered and showed few homologues in the trematodes. Similar to the ASBTs, CsASBT was predicted to have 10 transmembrane domains (TM) divided into two groups: a core group formed with TM3-5 and 8-10; a panel group formed with TM1, 2, 6 and 7. TM1-5 and TM6-10 were structurally homologous but oppositely oriented, thus producing an internal twofold pseudosymmetry. IF-CsASBT had three sodium binding sites. One binding site was coordinated by TM4b, TM5 and TM9a, and the other by TM3, TM4 and TM9. A third binding site was predicted to be coordinated by TM4a. Taurocholate binding pocket was located in an intracellular cavity. Structure-based virtual screening was carried out using a reliable IF- and OF-CsASBT model. Inhibitors, not working on human ASBT, were selected through a pharmacophore-based filtering. Taken together, we report the refined models of CsASBT important for further study on function of the ASBTs and on structure-based drug design targeting the ASBTs.

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OPISTHORCHIS FELINEUS HEMOZOIN DEPRESSES INTEGRIN CELL SURFACE EXPRESSION ON CHOLANGIOCYTES

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Infection with the fish borne liver fluke *Opisthorchis felineus* is common in the Eastern Europe (Ukraine, European part of Russia), Northern Asia (Siberia) and Central Asia (Northern Kazakhstan). The pathophysiology of the liver and bile duct tract due to parasitism coincides with the presence

of adult worms in the bile ducts, implicating worm excretory-secretory products acting on cholangiocytes, the epithelial cells lining the biliary tree. Excretory-secretory products of *O. felineus* include hemozoin, a byproduct of digestion of ingested host blood, which sequesters the toxic heme moiety. Hemozoin release from *O. felineus* accumulates in ectasia in the bile ducts -- hemozoin 'knobs'. We investigated the spread of hemozoin in the host circulation during distant organs during acute and chronic opisthorchiasis infection, and also the influence of hemozoin on cell growth. Using spectrophotometry and luminometry, we detected highest concentration of hemozoin in the bile ducts. Hemozoin was present in the parenchyma of the liver of hamsters during acute and chronic opisthorchiasis felinea, but not detected n heart, spleen, lung and muscles. The human cholangiocyte cell line H69 was exposed to hemozoin (O.F. hemozoin) isolated from bile ducts of hamsters, as well to synthetic hemozoin, and cell growth monitored in real time (xCELLigence System, Acea). O.F hemozoin lead to the changes of the adhesion characteristics of cells that dramatically decreased the cell index. By multiparametric flow cytometry, we found that both O.F and synthetic hemozoin depressed integrin beta-1, integrin beta-5 at the surface of H69 cells, and O.F hemozoin lead to the increasing of CD49a, an integrin alpha subunit. These findings indicated that liver fluke hemozoin induced raid and marked changes in the growth and adherence of cholangiocytes, findings that warrant further investigation.

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HYDROLOGICAL IMPACTS ON DISEASE TRANSMISSION OF *OPISTHORCHIS VIVERRINI* IN THE LAWA LAKE COMPLEX: A MODELLING PERSPECTIVE

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Several stages of *Opisthorchis viverrini*'s transmission cycle are mediated by water: from contaminated feces to snail as an egg, from snail to fish as cercariae, and from fish to definitive host as the infected fish is caught and consumed. All three of these processes are dependent on hydraulic connectivity, which changes seasonally in the floodplain ecosystem around the Lawa Lake complex in Khon Kaen Province, Thailand, our study site of interest. The prevailing theory, with limited empirical evidence to support, is that there are very limited circumstances and geographical areas in which these processes occur. Biology, parasitology, and ecology research inform us of some guiding assumptions that provide a tentative picture of how and where transmission is occurring in the environment. However, an exhaustive hydrologic model has never been developed and integrated into a transmission framework in order to explain the liver fluke development cycle. To that end, the development of a site-specific hydrodynamic model to understand connectivity between Lawa Lake, the Chi River, and surrounding wetlands is key to helping us identify "hot zones" of transmission, understand the scale of these transmission processes, and interpret historical and present data about infection levels in snails, fish, and humans. The model outputs flow vectors that elucidate the relationship between the villages around the lake, the scale of transmission, and proximity to susceptible habitats for snails and fish. The full model integrates the hydrologic model outputs as time-varying parameters into the disease model to describe transmission patterns. Results generated can be used to inform sustainable environmental control strategies in northeast Thailand and across Southeast Asia to reduce parasite and subsequent cancer burden.

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OPISTHORCHIS VIVERRINI INFECTION EXACERBATES THE SEVERITY OF DIABETIC LIVER INJURY AND NON-ALCOHOLIC FATTY LIVER DISEASE

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The highest co-prevalence between infection with *Opisthorchis viverrini* and diabetes mellitus (DM) is found in the northeastern Thailand. Inflammatory responses that lead to hepatobiliary disease is seen during opisthorchiasis. Moreover, non-alcoholic fatty liver disease (NAFLD) and diabetic liver injury caused by DM lead to the majority burden of liver disease at large. However, the association among opisthorchiasis, DM and hepatobiliary disease have not been clarified. The aim of these studies was to investigate the effect of *O. viverrini* infection on the development and progression of DM, NAFLD and diabetic liver injury in models using hamsters and cell lines. An experiment was carried out on four groups of hamsters: (1) normal control (NC), (2) chronic opisthorchiasis (OV), (3) NC and HFD fed with 10% fructose in drinking water for short-term (one month) and long-term (four months) (HF), and (4) *O. viverrini* infection at 4 months and followed by HFD fed with 10% fructose in drinking water for one and four month(s) (OVHF). Hamsters were euthanized at designated time points at five and eight months following infection. The fasting blood glucose levels of all experimental groups did not differ significantly. Intriguingly, the homeostatic model assessment of insulin resistance (HOMA-IR) in short-term HF treatment of group 3 was higher than other groups at the same time point. However, the HOMA-IR of long-term HF treatment of group 3 was not different from OVHF group but higher than OV group at the same time point. Histological features of hamster livers revealed that the highest amount of lipid droplet was found in short-term HF treatment of OVHF group. In addition, we observed that excretory/secretory products of *O. viverrini* suppressed the growth of HepG2 cell line by dose-dependent manner when monitored under real-time monitoring system. These findings indicate the effect of *O. viverrini* on the improvement of insulin sensitivity. Infection with *O. viverrini* might inhibit regeneration of hepatocytes and increase the severity of diabetic liver injury, and hence chronic opisthorchiasis might represent a risk factor for NAFLD.

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DEVELOPMENT OF A NOVEL METHOD FOR *OPISTHORCHIS VIVERRINI* DNA DETECTION IN URINE BY PCR ASSAY

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Opisthorchis viverrini infects 9 million people worldwide and is endemic in Thailand, Laos, Vietnam and Cambodia. Treatment is the most effective tool we have to reduce the transmission and burden of disease while increasing the quality of life for persons infected. In order to effectively treat, we must diagnose those who are infected. Microscopy is the current diagnostic standard for *O. viverrini* through the identification of eggs in stool but is hampered by under diagnosis and misclassification. Our goal is to develop a protocol for *O. viverrini* DNA detection in urine for future diagnostic purpose. We developed and validated an extraction technique to isolate *O. viverrini* DNA from urine using BioMatrix Resin by adapting a method recently developed for *Schistosoma mansoni*. PCR, using *O. viverrini* -specific primers (pOV-6) was performed and optimized to detect the isolated DNA. 5ml of clean negative human urine was spiked

with 3500 ng of *O. viverrini* DNA and a serial dilution was performed to determine the detection limit. A 5ml pooled sample of 10 positive human samples was made and we performed a serial dilution to determine the profile of O.viverrini DNA by PCR. The PCR using pOV-6 primers on DNA extracted from urine worked successfully in spiked samples and human samples. The extraction method resulted in the retention of 33% of the original DNA amount. The detection limit of *O. viverrini* DNA in urine is 0.077 pg. The pooled samples showed DNA presence at a 10-25% dilution but not at higher or lower concentrations. This is the first report of the detection of *Opisthorchis viverrini* DNA in urine. Urine is easier to obtain from humans than feces or blood samples because of cost, time, and intrusiveness. Overall we were able to achieve the detection of *O. viverrini* DNA in urine, which prompts potential investigation as to why the DNA is there and allows for further develop a sensitive and specific diagnostic assay for opisthorchiasis with the goal of reducing morbidity and mortality.

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MOLECULAR CHARACTERIZATION OF THE LARVAL PHASE OF *SCHISTOSOMA MANSONI* IN *BIOMPHALARIA GLABRATA* MOLLUSKS UNDER EXPERIMENTAL CONDITIONS

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Schistosomiasis mansoni affects approximately 207 million people in the world. In Brazil, a major goal of the Control Program for this parasitosis is to reduce the risk of geographic expansion. The main intermediate host of *Schistosoma mansoni* is *Biomphalaria glabrata*. The detection of larval stages in intermediate hosts is an important challenge to public health once it can indicate early natural infections rates. The objective of this study is to standardize the detection of *S. mansoni* from primary sporocysts developed in *B. glabrata* mollusks tissue artificially infected with Belo Horizonte *S. mansoni* lineage, using the Polymerase Chain Reaction (cPCR), a Two Sequential PCR-amplification (Re-PCR) and TaqMan® Real-Time PCR system (qPCR). Twenty *B. glabrata* mollusks were infected with thirty miracidia obtained from a laboratorial cycle of *S. mansoni*. For *S. mansoni* DNA extraction, after thirty days, four daughter-sporocysts were collected. Moreover, the head-foot portion was removed from four additional specimens of *B. glabrata*. The nucleic acid was extracted using the DNeasy Blood and Tissue Kit (Quiagen). Extracted DNA was quantified using Nanodrop and amplified with the three molecular techniques cited above, using primers that amplify 121bp *S. mansoni* DNA tandem sequences. The amplification product was detected in agarose gel 2%. The extraction process of daughter-sporocysts yielded around 6.1 ng/µL of *S. mansoni* DNA. The amplification products of cPCR were faint, but the results were better after Re-PCR and qPCR, which showed a mean of 20.07 Cycle Treshold (Ct). *S. mansoni* DNA extraction from complete head-foot portion yielded an average of 487.5ng/µL, with good results in cPCR, Re-PCR and qPCR that pointed a Ct average of 15.92. *S. mansoni* sporocyst molecular detection from the head-foot portion demonstrated a high sensibility and specificity. Therefore, it could represent a new tool in early characterization of snail susceptibility to *S. mansoni* in natural conditions and assist in the control of this parasitosis.

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IDENTIFICATION OF GENES TARGET OF REGULATION BY MAPKS IN *SCHISTOSOMA MANSONI*

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Eukaryotic protein kinases (ePKs) are important for the regulation of several cellular functions. It is known that 252 ePKs are encoded by the *Schistosoma mansoni* genome, which corresponds to 2% of the predicted proteome. However, only 24 ePKs have experimental functional evidence. The family of mitogen-activated protein kinase (MAPKs) influences various biological activities and are widely studied as drug targets. Our group identified MAPKs orthologs in *S. mansoni* by *in silico* analyses and demonstrated by functional studies that MAPKs (SmCaMK2, SmJNK, SmERK1, SmERK2 and Smp38) are involved in parasite development, reproduction and survival and may therefore be considered potential targets for the development of new drugs. In this study, we aim to contribute to the experimental characterization of ePKs by the identification of specific genes regulated by MAPKs pathways. To elucidate these target genes, the five selected genes were knocked down by RNA interference in schistosomula and RNA-Seq analysis of treated parasites were performed, including three biological replicates. For all genes selected, we observed approximately 75% reduction on transcript levels, except for SmERK-2. RNA-Seq libraries were then prepared with RNA derived from knockdown parasites according to the *Truseq stranded mRNA Library Prep* protocols and were sequenced on *Illumina HiSeq 2500* platform. We generated 27 paired-end libraries that generated 100 bp reads. Sequences were aligned to the latest *S. mansoni* reference genome (version 5.0) and differentially expressed genes were identified by comparing each MAPK knockdown against control parasites (treated with unspecific GFP-dsRNA). We also checked the occurrence of potential off-targets by using GFP or mCherry dsRNA as unspecific controls. RNA-seq analyzes were performed comparing schistosomules treated with these unspecific dsRNAs and schistosomules untreated. This work will allow a better understanding of these signaling pathways, helping to elucidate the functional roles of MAPKs, as well as assisting in the future development of therapeutic intervention tools for schistosomiasis control.

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HISTONE MODIFYING ENZYMES ARE POTENTIAL THERAPEUTIC TARGETS AGAINST SCHISTOSOMIASIS AS IT IS ESSENTIAL TO VIABILITY AND REPRODUCTION

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Schistosomiasis is the second most prevalent parasitic disease in the world. The treatment rely on a single drug, Praziquantel, and due to the identification of drug resistant parasites, the development of new chemotherapy against schistosomiasis is required. Histone modifying enzymes (HMEs) play a central role in regulating chromatin epigenetic modifications and are implicated as therapeutic targets in various diseases. In this work, we employed RNA interference to validate 1 histone deacetylases (SmHDAC8), 5 demethylases (HDM) and 10 methyltranferases (HMT) as drug targets in *Schistosoma mansoni*, from those, 4 were chosen for experimental validation. Additionally, specific inhibitors developed by the A-PARADDISE consortium were used to interrogate HMEs as drug targets against *S. mansoni*. To elucidate the roles of HMEs, schistosomula were exposed to dsRNAs, injected in mice and evidenced that SmHDAC8 is important to parasite development and survival. Additionally, HDAC8,

PRMT3 and KDM1/KDM2, seem to be associated in egg production since infected mice had significantly lower egg burdens and female worms presented underdeveloped ovaries. For the inhibitors screening, schistosomula were exposed to 300 inhibitors and parasite viability was assessed by measurement of lactate produced in the medium and by propidium iodide staining. These inhibitors were also tested on adult worms, in which parasites mobility were evaluated using the WormAssay software. In the lactate and propidium iodide assays, 60 and 76 active inhibitors were identified, respectively. Using the WormAssay, 76 compounds were active in male worms, 101 were active in female worms, from those, 54 were active in both genders. Also, the IC50 was established for the active compounds and cytotoxicity was tested in mice fibroblast cells. These results indicate that HMEs are essential to parasite viability, oviposition and/or development of reproductive system, confirming its potential as drug targets. In addition some inhibitors seem to be potential candidates for drugs against schistosomiasis.

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CLONING AND CHARACTERIZATION OF A *SCHISTOSOMA JAPONICUM* AQUAGLYCEROPORIN THAT FUNCTIONS IN OSMOREGULATION

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As one of the three major human pathogens that cause schistosomiasis, *Schistosoma japonicum* is the only one that is endemic in China. Despite great progress on schistosomiasis control over the past 50 years in China, *S. japonicum* transmission still occurs in certain endemic regions, which causes significant public health problems and enormous economic losses. During different life stages, parasites are able to survive dramatic osmolality changes between its vector, fresh water, and mammal host. However, the molecular mechanism of parasite osmoregulation remains unknown. To address this challenging question, we report the first cloning of an *S. japonicum* aquaglyceroporin (SjAQP) from an isolate from Jiangsu province, China. Expressing SjAQP in Xenopus oocytes facilitated the permeation of water, glycerol, and urea. The water permeability of SjAQP was inhibited by 1 mM HgCl2, 3 mM tetraethylammonium, 1 mM ZnCl2, and 1 mM CuSO4. SjAQP was constitutively expressed throughout the *S. japonicum* life cycle, including in the egg, miracidia, cercaria, and adult stages. The highest expression was detected during the infective cercaria stage. Our results suggest that SjAQP is very likely to play a role in osmoregulation throughout the *S. japonicum* life cycle, especially during cercariae transformation, which enables parasites to survive osmotic challenges.

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THERAPEUTIC EXPLOITATION OF IPSE, A UROGENITAL PARASITE-DERIVED HOST MODULATORY PROTEIN, FOR CHEMOTHERAPY-INDUCED HEMORRHAGIC CYSTITIS

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The vast majority of urogenital schistosomiasis-infected individuals lack day-to-day hematuria, a cardinal sign of urinary tract injury. This suggests that parasite factors may help balance life cycle propagation via bladder wall penetration to allow egg passage into urine, against inducing life-threatening hemorrhagic cystitis (HC) that would preclude life cycle completion. IL-4-inducing principle from *Schistosoma mansoni* eggs (IPSE) is the most abundant egg-secreted protein of *S. mansoni*, which induces IL-4 release from basophils via binding to cell-surface IgE. IPSE also sequesters chemokines and alters host gene transcription by translocating into host nuclei. We hypothesized that the *S. haematobium* homolog of IPSE (H-IPSE) may regulate HC. Because schistosome transgenesis remains elusive, we tested host-modulatory properties of H-IPSE using a model of HC based on nitrogen mustard alkylating agents (cyclophosphamide and ifosfamide), which are used to treat cancers but often result in HC. Current options to prevent ifosfamide-induced HC using Mesna can have significant side effects. IL-4 has been shown to ameliorate HC in mice, but systemic IL-4 administration results in unacceptable morbidity. Given the IL-4-inducing properties of IPSE, we postulated that H-IPSE may ameliorate ifosfamide-induced HC. Mice were administered combinations of ifosfamide, IL-4, Mesna, anti-IL-4 antibody and H-IPSE. Readouts include bladder wet weight, histology (i.e., edema, hemorrhage), and hemoglobin content, spontaneous and evoked pain, voided urine spot assay, cytokine analysis and transcriptional profiling. We found that H-IPSE is comparable, and possibly superior, to IL-4 in suppressing ifosfamide-induced HC in mice, including associated urinary frequency and spontaneous pain behavior. Through use of anti-IL-4 antibody and a nuclear localization sequence mutant of H-IPSE, we determined that the therapeutic effect was dependent on IL-4 and nuclear localization. To our knowledge, our work is one of the first successful therapeutic exploitation of uropathogen-derived molecule in a clinically relevant bladder disease model.

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SCHISTOSOMA HAEMATOBIUM IPSE, A CANDIDATE PRO-ONCOGENIC FACTOR

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Urogenital schistosomiasis (UGS) affects over 112 million people globally. Adult worm pairs in the pelvic venous plexus deposit eggs in the bladder. The eggs secrete antigens that induce granuloma formation, in turn provoking immunopathogenic sequelae that include urothelial hyperplasia and carcinogenesis. The IL-4-inducing principle of *Schistosoma mansoni* eggs (IPSE) is a prominent antigen released by schistosome eggs. IPSE binds immunoglobulins and chemokines, translocates into host nuclei and modulates gene transcription, and induces basophils, mast cells, and NK T-cells to release IL-4, thereby orchestrating a dominant Th2 response. Given that the IPSE gene is only found in Schistosoma, it is a candidate pro-carcinogenic factor in schistosomal bladder cancer. We hypothesize that the *Schistosoma haematobium* homolog of IPSE (H-IPSE) plays a major role in driving the inflammation-associated urothelial proliferation and bladder carcinogenesis during UGS. Recombinant H-IPSE was co-cultured with a panel of urinary bladder cell lines derived from primary and transformed tissues, representing diverse species of origin and stages of carcinogenesis: HTB-9 (Grade II human bladder carcinoma), HCV-29 (from normal human bladder urothelium) and MB49 (mouse urothelial carcinoma). Readouts included CFSE proliferation assays, cell cycle analysis, TUNEL apoptotic assays, real-time monitoring of cell migration and invasion using the xCELLigence platform, and qPCR-based transcriptional profiling. Whereas an influence of H-IPSE on proliferation and apoptosis of HTB-9 cells was not seen, it stimulated proliferation of HCV-29 and MB49 cells in a concentration-dependent manner. Moreover, cell cycle analysis showed that H-IPSE increased the proportions of MB49 cells in the S-phase. These findings indicate that H-IPSE might contribute to bladder cancer progression in the context of UGS. Ongoing work will define the mechanisms by which H-IPSE promotes *S. haematobium*-associated oncogenesis.

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PROTON CHANNELS IN *BIOMPHALARIA GLABRATA* EMBRYONIC CELL MEMBRANES: PUTATIVE TARGET FOR *SCHISTOSOMA MANSONI* LARVAL TRANSFORMATION PROTEINS

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The *Biomphalaria glabrata* embryonic (Bge) cell line was derived from the freshwater snail *B. glabrata,* an intermediate host for *Schistosoma mansoni*, a causative agent of intestinal schistosomiasis. Bge cells share characteristics with hemocytes, the immune effector cells of *B. glabrata* snails, and therefore, were used as an *in vitro* model for the study of host cell-larval parasite interactions. This study employed the whole-cell patch clamping technique to identify the major ion channel channels in the cell's plasma membrane and to explore their possible role in host-parasite interactions. Bge cells were exposed to pH gradients that resulted in changes in proton channel current. Exposure to Zn^{2+}, a potent proton channel blocker, reduced response amplitude in Bge cells by 3-fold, further supporting the presence of proton channel activity. A series of voltage steps (-70 mV to 20 mV) applied to Bge cells evoked current responses that were significantly enhanced by *S. mansoni* larval transformation proteins (LTP), indicating an LTP-modulation of these channels. Since proton channels can play a role in the production of reactive oxygen species (ROS) in mammalian immune cells, we tested this function in Bge cells. Using the fluorescent probe 2',7'-dichlorofluorescein-diacetate (DCFH-DA) to detect intracellular ROS, we found that cells generated an ROS response that was significantly reduced by Zn^{2+}, indicating the involvement of proton channels in ROS production. LTP had no significant effect on ROS production suggesting that LTP-induced flux increases through proton channels may not extend to its modulation of the oxidative response. Lastly, immunofluorescence analyses of Bge cells and *B. glabrata* hemocytes revealed the expression of a HVCN1-like proton channel demonstrating that *B. glabrata* hemocytes also express a proton channel that may mediate ROS production. Thus, this channel could be important for the killing of larval *S. mansoni* by hemocytes of resistant *B. glabrata* snail strains.

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CHARACTERIZATION AND FUNCTIONAL STUDIES OF SERINE/THREONINE PROTEIN PHOSPHATASE 1 (PP1) ENCODING GENES FROM *SCHISTOSOMA JAPONICUM*

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Serine/threonine protein phosphatase1 (PP1) play a wide range of physiological roles including cell cycle regulation, glycogen metabolism, contractility, morphogenesis and spermatogenesis. However, little is known about the functions of PPs in the reproductive biological processes of schistosomes. In the present study, three PP1 genes were identified in *Schistosoma japonicum*. The sequence alignments and phylogenetic analyses showed that three PP1 proteins (Sj-PP1-1, Sj-PP1-2 and Sj-PP1-3) belong to PP1 beta, PP1 gamma and PP1 alpha subfamily, respectively. RT-PCR analysis revealed that three PP1 genes were all transcribed in both sexes and throughout development. In-situ hybridization indicated that Sj-pp1 were predominantly expressed in gonad related organs such as the testis of male, the ovary and vitellarium of female as well as ootype surrounding area. RNA interference with combined three Sj-PP1 dsRNAs by soaking for 7d caused stunted growth of female and male worms. CLSM observation found distinct morphological changes in Sj-PP1 dsRNA treated female worms with significantly smaller ovaries which were dominantly occupied by immature oocytes and low maturity of vitellarium surrounding by immature vitelline cells. In addition, no significant changes were observed on males treated for 7d, except for a reduced diameter of the testicular lobes accompanied by a reduction of cell density in testes and empty seminal vesicles with prolonged RNAi by 12d. Edu corporation assay detected evident decrease of cellular mitosis activities in ovary, vitellarium of females and testis and parenchyma of males. With extension of RNAi process, remarkable reduction in egg production were observed and serious interference on paring behavior was found between female and male. These findings suggested that PP1 may function in developmental and reproductive processes of *Schistosoma japonicum*.

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INTERACTIONS BETWEEN HOST IMMUNE STATUS AND PARASITE METABOLIC ACTIVITY IN *SCHISTOSOMA MANSONI*

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Schistosomes are obligate parasites that lack genes required for the synthesis of many lipids (i.e. cholesterol and long-chain fatty acids), and therefore rely solely on the mammalian host to supply these essential molecules. Lipid acquisition appears to be particularly important for female schistosome reproductive activity. Here we show that lipid is specifically concentrated in the ovary of the female parasite. Interestingly, reproductively inactive females, in both unisexual infections and immunodeficient hosts, exhibit reduced accumulation of lipid in the ovary, underlining the connection between lipid metabolism and parasite reproduction. Coincident with these changes in lipid accumulation, schistosomes from immune competent and immunodeficient hosts also exhibit alterations in ATP metabolism. At 6 weeks post-infection, schistosomes isolated from immunodeficient animals have significantly higher ATP content than those isolated from immune competent animals. However, at 8 weeks post-infection the situation is reversed, with schistosomes isolated from immunodeficient animals having significantly lower ATP content than those isolated from immune competent animals. These findings suggest that parasites in immunodeficient hosts are unable to synthesize sufficient ATP once egg production is underway. To explore the molecular pathways connecting parasite lipid acquisition, energy metabolism, and reproduction, we are characterizing the parasite [mTOR] and [AMPK] signaling pathways to assess their status in these different developmental states. These findings suggest that a link between host immune status and parasite energy metabolism is an important aspect of the host-schistosome relationship.

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DUAL RNA-SEQ RESPONSES OF FIELD-DERIVED SPECIMENS OF THE AFRICAN SNAIL *BIOMPHALARIA PFEIFFERI* TO INFECTION WITH THE HUMAN PARASITE, *SCHISTOSOMA MANSONI* PROVIDE INSIGHT INTO HOST-PARASITE RELATIONSHIPS AND REPRODUCTIVE IMPLICATIONS OF PARASITISM

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Biomphalaria pfeifferi exhibits extraordinary compatibility with *Schistosoma mansoni* and likely transmits more cases of this parasite to people than any other snail species. Ironically, we know relatively little at the molecular level regarding the interactions of *B. pfeifferi* and *S. mansoni* from early-stage sporocyst transformation to the development of cercariae. To redress this shortcoming, using field-derived west Kenyan representative of schistosomes and snails, we have undertaken dual RNA-seq of three intramolluscan developmental stages (1- and 3-days post exposure and cercariae producing infections). Parasite sequences were first separated by screening against the *S. mansoni* genome. Then, a *de novo B. pfeifferi*

transcriptome was assembled from over a half billion non-*S. mansoni* paired-end reads. Transcripts were annotated using protein and nucleotide databases, including the *B. glabrata* genome database. Snail reproductive inhibitory peptides like ovipostatin and developmentally regulated albumen gland protein were up-regulated in shedding snails, suggesting that host castration is not merely a passive response to diminished energy supplies. The lack of expression of snail sex pheromones may be related to the strong self-fertilizing preferences of *B. pfeifferi*. Fibrinogen-related proteins (FREPs) showed complex patterns of responses. Distinctive profiles of expressed *S. mansoni* features were seen, including up-regulation of defense and stress response proteins. Five *S. mansoni* venom allergen-like proteins, known for host immunomodulatory functions, were highly up-regulated in shedding snails. These field-derived snails harbored several notable symbionts including *Capsaspora owczarzaki*, microsporidians, *Perkinsus*-like protists, and ectosymbionts like *Trichodina* and *Chaetogaster*. Our database provides unique insights into schistosome-snail interactions taking place in a natural transmission focus, potentially including candidate molecules amenable to manipulation to facilitate new control approaches targeting the ability of larval schistosomes to succeed in their snail hosts.

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SEX-BIASING GENE DRIVE TO ELIMINATE SCHISTOSOMES: A PROPOSAL

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Sex-biasing gene drives ensure that nearly all offspring are of one sex in order to progressively reduce the reproductive potential of the population. One strategy confers a fitness advantage to a sex chromosome by shredding the "opposing" sex chromosome during meiosis such that the driving chromosome is preferentially inherited by offspring. We propose a population suppression drive intended to locally or globally eradicate the schistosomes. Draft versions of the genome sequences of *Schistosoma mansoni*, which causes hepato-intestinal schistosomiasis, and *S. haematobium*, the cause of urogenital schistosomiasis, are available. Because the genetics and chromosomal architecture of *S. mansoni* are better understood, we are focusing on *S. mansoni* and will follow with *S. haematobium* once comparable information is available. We propose to deploy CRISPR/Cas9- and pseudotyped retrovirus–based techniques to introduce the gene drive components into the schistosome germ line. Schistosomes are a ZW species; females are ZW, males ZZ. The Z and W are largely homologous, but there are unique regions of both. It is therefore feasible to make a female-biasing 'Z-shredder' and a male-biasing 'W-shredder'. From a fitness perspective the Z-encoded W-shredder may be superior because it can also act as a conventional drive in ZZ males. That is, in ZW females it will shred the W, thereby ensuring that offspring inherit the driving Z chromosome and are consequently male, but will also copy itself from the driving Z to the wild-type Z in heterozygous males. Both activities are advantageous and should consequently be evolutionarily favored over time, albeit opposed by any unlikely suppressors that might evolve on the W or autosome. The gene encoding ribosomal protein S4 resides on Z, is single copy, and is expected to be critical for survival, while numerous repeats on the W are suitable for evolutionarily stable shredding. We are assaying components for building drive systems and are optimistically assembling and testing a W-shredder rps4 drive; and as a safeguard, an immunizing reversal rpS4 drive. Our proposal, approach and preliminary findings will be discussed.

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ADVANCING WATER TREATMENT FOR RESOURCE RECOVERY TO ENHANCE DISEASE MITIGATION

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Despite being a fundamental resource required for the sustainment of life, water plays an integral part in the lifecycle and transmission of many pathogens, including bacteria, viruses, and parasites. This dichotomy presents an increased risk of exposure to a wide range of pathogens for humans and animals living in regions without regular access to clean water and waste processing systems, such as flush toilets. A large number of the technologies that have been designed solely for water and waste treatment fail in the field because they are not economically viable, whether through initial costs or long-term maintenance, or they have not been designed to withstand the unique environmental or cultural challenges presented by differing regions of application. In order to eliminate water as a reservoir for disease transmission, technologies designed for water treatment need to provide effective filtration or sterilization while allowing for the recovery of valuable resources. Examples of such resources may include, but are not limited to fertilizer, harvestable energy, and chemical components that may lead to the production of larger commodities. By providing waste and/or water treatment to reduce the transmission of disease while recovering harvestable products, the cost of initial construction and maintenance on such systems can me offset by the sustainability of the system and the potential for economic stimulation through the creation of technical maintenance jobs and new trade industries. This study will review a number of currently available technologies, describe the advantages and challenges for each technology, and discuss the potential for adaptation for use in resource-limited environments.

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PREVALENCE OF SOIL TRANSMITTED HELMINTHS IN WATER, SANITATION AND HYGIENE (WASH) SUPPORTED AND NON-SUPPORTED SCHOOLS IN OGUN STATE, NIGERIA

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Water, Sanitation, and Hygiene (WASH) interventions have been advocated as a complementary tool in the control of soil-transmitted helminths (STH) infection. We therefore assessed the prevalence of STH in WASH supported and non-supported schools in Odeda, a rural local government area of Ogun state, Nigeria. Eight schools were randomly selected across the study area; three WASH supported (S-schools) and five non-supported schools (NS-schools). Stool samples were collected from 428 pupils and screened for STH infection, followed by an assessment of school-based WASH resource using WHO recommended guide. Results showed significant differences ($p < 0.05$) in the provision of safe water and environmental hygiene for S-schools (100% and 73.3%) and NS-schools (16% and 26.9%) respectively. There exist no significant differences ($p > 0.05$) in the sanitation condition between S-schools (44.4%) compared to NS-schools (20.0%). Overall prevalence of STH infection was 33.4%, while specific prevalence of 26.2% was recorded for Hookworm, 18.2% for *Ascaris lumbricoides* and 1.6% for *Trichuris trichiura*. STH Prevalence were significantly lower ($p<0.05$) in S-schools (27.4%) compared to NS-schools (37.5%). Mean intensities of hookworm and *Trichuris trichiura* infections were higher in NS-schools than S-schools (0.6097epg and 0.4247epg for hookworm) and (0.1193epg and 0.0epg for *Trichuris trichiura*) respectively.

This study provides evidences that WASH interventions have the potentials of reducing intestinal helminthiasis burden in public primary schools and should be scaled up to include more public schools in the state.

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WATER SUPPLY AND SANITATION CONDITIONS IN RURAL SOUTHERN MOZAMBIQUE AND ITS AS SOCIATION WITH MORBIDITY AND MORTALITY INDICATORS DURING 2012-2015

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Water, sanitation and hygiene (WASH) are major health determinants, with an estimated total disease burden of 5.7% occurring worldwide.The situation of access to safe water and sanitation facilities and its impact on morbidity and mortality in southern Mozambique remains unknown. The aim of this study is to describe the current situation of safe water supply and sanitation facilities in the Manhiça Health Research Centre (CISM) study area and evaluate its association with several morbidity and mortality indicators. We conducted a retrospective cohort study with 61,900 children living in the center study area followed up until 15 years of age during the period 2012-2015. Water and sanitation household data was obtained from the CISM demographic surveillance system in Manhiça district, an area of around 2,380km2. Clinical data for all children under 15 was obtained from CISM round-the-clock morbidity surveillance system covering pediatric outpatient and hospital admission at the Manhiça District Hospital and rural health posts. A negative binomial regression model using Wald test was performed to assess the incidence rate ratio for every morbi-mortality indicator. Preliminary data showed that 86% of the children lived at least once in a household with unimproved sanitation facilities, 27% with unimproved water source and 77% with the main water source located outside the household during 2012-2015.Unexpectedly, the incidence rate ratios to develop diarrhea for children using unimproved sanitation and water facilities were significantly protective. Only the use of rivers and lakes as water sources significantly increased the children's rate to develop diarrhea by 20%. Other morbidity indicators (malnutrition, parasitemia, anemia) did show a rate increase with the use of unimproved water and sanitation facilities. Spatial distribution and clustering for the water, sanitation and morbidity variables will be also analyzed.The possible explanation of the findings will be discussed.This analysis will help to plan evidence-based interventions to improve access to safe drinking water and sanitation in rural southern Mozambique.

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ASSOCIATION OF HOUSEHOLD, COMMUNITY AND SCHOOL SANITATION WITH HOOKWORM INFECTIONS AMONG SCHOOL-AGED CHILDREN IN KWALE COUNTY, KENYA

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Most of our understanding of the association between sanitation and STH infection is focused separately on schools and households, and few studies simultaneously assess the relative importance of sanitation in the household, wider community, and schools. Yet, understanding the relative impact of sanitation on STH transmission between these domains is necessary to target disease control interventions for greatest impact. Here we investigate associations and interactions between hookworm infection and household, community, and school sanitation among children aged 5-14 in Kwale County, Kenya. Data were collected during a cross-sectional parasitological survey between March-May 2015 as the baseline for the TUMIKIA study. Data were available from 22,864 households randomly selected across the county using a two-stage sampling design. In each household, one member was randomly selected and invited to provide a stool sample. Sanitation was assessed using structured observations and questionnaires. Sanitation conditions for every school in Kwale County were assessed during a survey in June 2015. Records from school and household surveys were linked for each child. Generalized linear mixed models were used to estimate the association between measures of household, community, and school sanitation with presence and intensity of hookworm infection. In total, the analysis included 5,251 school-aged children in 841 villages. Overall hookworm infection prevalence was 17.4% (16.4--18.4%), while mean intensity was 156.2 epg (141.4--172.6). Household sanitation coverage was estimated, as the proportion of households with reported access to a toilet, to be 49.2% (47.9--50.6%), and village sanitation coverage had an IQR of 25.0--78.4%. Multilevel analysis revealed associations across various domains, highlighting important areas of exposure for school-aged children in endemic communities. This study contributes further to our understanding of the impact of sanitation on hookworm infection in respective domains, and provides insight into effective targeting of programs to maximize reductions in hookworm infection.

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THE DRIVERS OF THE CHOLERA EPIDEMIC IN BAUCHI, NORTHEAST NIGERIA 2014

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On 19th February, 2014, the Nigerian Federal Ministry of Health (MOH) was alerted of an ongoing outbreak of cholera in Bauchi State, Nigeria. The State has experienced repeated cholera outbreaks almost yearly with the 2010 outbreak, recording one of the highest case burdens among the states in Nigeria. We investigated the outbreak to assess the magnitude of the outbreak and identify risk factors for transmission. We collected the line-list from the State MOH and conducted a case-control study. We collected data on demographic characteristics, hygienic practices, and on food and water consumption using a structured questionnaire. We identified 2998 cases among the 1,444,393 residents of the 4 affected Local Government areas. Overall attack rate and case fatality rate were 0.21% and 0.77% respectively. Among the 248 case-control study

participants, 113 (45.6%) were female and 135 (54.4%) were male. There were 124 cases and 124 controls. Compared to controls, cases were more likely to have been exposed to diarrhoea case (OR:6.72, 95% CI 3.87-11.75), live as an Islamic Mendicant 'almajiri' (OR:5.22, 95%CI 1.94 - 14.78), not wash hands with soap after toileting (OR:4.71, 2.59 - 8.62), not wash hands before eating (OR:3.82, 95%CI 1.13 - 14.20), be ≤25 years (OR:3.32, 95%CI 1.97 - 5.60), drink street vended locally processed cereal drink 'kunu zaki' and custard 'koko' (OR:3.28, 95%CI 1.15 - 9.31) and (OR:2.91, 95%CI 1.10-7.71) respectively. In conclusion, 'Almajiris' were found to be key players boosting the epidemic and consumption of food from street vendors was a major risk factor for the spread of the disease. Sanitary inspection officers should mobilize the 'Almajiris' to maintain clean environment around them and food hygiene. The health department should train hawkers of foods on the street on personal and food hygiene.

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PREVALENCE OF ROTAVIRUS INFECTION OVER TIME IN RURAL, COASTAL ECUADOR

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Rotavirus is a key cause of diarrheal disease in the developing world. However, patterns of infection over time have not been well characterized. Using 10 years of population based case-control data from rural coastal Ecuador, we investigate the prevalence of rotavirus infection over time and differences by subgroup in a region that has been experiencing continuous road construction. The prevalence of rotavirus infection has steadily declined over time from 7.0% in 2003 to 1.3% in 2012. This decrease was 3.4 times faster for symptomatic infection (rates of 1.3% and .4% respectively). Rotavirus infection was highest among individuals under age 5 (OR=3.19, 95% CI: 2.58, 3.94) but all age groups except children under 1 year of age exhibited decreases in prevalence of infection over time. In contrast, younger children showed patterns characteristic of a long epidemic, with a peak infection risk of 16.7%. Despite their higher levels of exposure, household and community controls had similar prevalence of infection (p=.82), suggesting that community interactions may be important for transmitting asymptomatic rotavirus infection in our study region.

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A RANDOMIZED CONTROLLED TRIAL OF A HOSPITAL-BASED HANDWASHING WITH SOAP AND WATER TREATMENT INTERVENTION (CHOBI7) TO REDUCE CHOLERA AMONG HOUSEHOLD CONTACTS OF CHOLERA

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Household contacts of cholera patients are at a 100 times higher risk of developing a cholera infection than the general population during the week post-presentation of the index patient at the hospital. In an effort to initiate a standard of care for highly susceptible household contacts of cholera patients, we developed a hospital-based handwashing with soap and water treatment intervention entitled CHoBI7(Cholera-Hospital-Based-Intervention-for-7-days). The CHoBI7 intervention includes: (1) a cholera prevention package containing chlorine tablets for water treatment, soapy water bottles, a handwashing station, and a sealed water vessel with cover to ensure safe water storage, and (2) a pictorial ("Chobi" in Bangla) module on handwashing with soap and water treatment delivered by a health promoter through hospital and home visits during the week post-presentation of the index cholera patient at the hospital. The efficacy of the CHoBI7 intervention was evaluated by conducting a randomized controlled trial of 219 intervention household contacts of cholera patients and 220 control contacts of patients in Dhaka, Bangladesh. Case households were followed over a one week period at 5 timepoints for clinical and environmental surveillance which included collection of rectal swab and source and stored water samples to test for the presence of *Vibrio cholerae* by bacterial culture. Five hour structured observation was also conducted to assess handwashing practices. Compared to control contacts, intervention contacts had a significant reduction in symptomatic cholera infections (OR: 0.00 (95% CI: 0, 0.623)), and a 47% reduction in overall cholera infections (OR: 0.50 (95% CI: 0.21, 1.18). Intervention households had no stored drinking water with detectable *Vibrio cholerae*, and a 14 times higher odds of handwashing with soap at key events than control households during the intervention period (OR: 14.68 (95% CI:8.32, 25.90)). In conclusion, the CHoBI7 intervention presents a promising approach for cholera control among highly susceptible household contacts of cholera patients.

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THE ASSOCIATION BETWEEN HEAVY RAINFALL EVENTS AND DIARRHEAL DISEASE: THE INFLUENCE OF URBAN AND RURAL GEOGRAPHY

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Climate change is expected to have downstream impacts on health outcomes in the 21st century. Changes in precipitation are expected with greater contrasts between wet and dry periods and increase in extreme weather events. Heavy rainfall events (HRE) have been shown to be associated with diarrheal disease. Diarrheal disease remains an important cause of mortality amongst children under five years of age with causing over 700,000 deaths per year and is associated with long term health outcomes such as stunting. Differences in urban and rural settings could play an important role in driving the relationship between precipitation and diarrhea due to underlying differences in infrastructure and social factors. The study aims to analyze the role of urban and rural contexts in affecting the relationship between HRE and diarrheal disease. Mixed effects Poisson regression was conducted on daily case counts of diarrhea in all public hospitals and clinics from all 68 parishes in the Esmeraldas province in northwestern coastal Ecuador across 2013-14 with heavy rainfall estimates and antecedent conditions lagged up to 14 days. Average daily rainfall estimates from the TRMM 3B42 platform were used to define heavy rainfall events as daily rainfall higher than the 90th percentile and antecedent conditions as wet or dry depending on 8 week total rainfall being in the highest or lowest tertile for the respective parish. In rural areas, there was a protective effect of HRE on daily case counts of diarrhea during the wet season whereas a positive association was observed in the dry season. In urban areas there was no protective effect observed and the expected counts of diarrhea were higher in all environmental conditions analyzed when compared to rural areas in the wet season. Factors associated with urbanization, such as crowding or infrastructure, seem to dominate over climate related factors. Despite this, dry conditions appear to be highly associated with increased diarrhea in all areas. Further work is needed to elucidate the mechanistic structure of what factors are driving the differences between urban and rural areas in how rainfall is associated with diarrhea.

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MULTI-SECTORAL COLLABORATION BETWEEN THE NTD AND WASH SECTORS: EXPERIENCE FROM UGANDA

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In order to eliminate blinding trachoma all components of the SAFE strategy (Surgery, Antibiotic, Facial cleanliness and Environmental improvement) must be in place; however, the F & E components are often given less attention and financial resources. Many national programs are eager to implement F & E activities but do not know where or how to start. In Uganda, the Ministry of Health (MOH), in partnership with the Ministry of Water and Environment, Ministry of Education and Sports, various Water, Sanitation and Hygiene (WASH) and trachoma organizations came together to develop a comprehensive F & E plan. This process involved conducting an F & E situational analysis, organizing multiple multi-sectoral meetings and reaching a consensus on program priorities. Ultimately, four main activities were identified: 1) integration of face washing and trachoma messages into existing WASH strategies and activities in trachoma endemic regions; 2) revision and dissemination of school sanitation guidelines; 3) revision and dissemination of national sanitation guidelines; and 4) development of a Social and Behavior Change Communication (SBCC) strategy to be used as part of mass media campaigns. A review committee composed of representatives from different government ministries and organizations evaluated different WASH organization's proposals and selected the best proposal for each activity. As of December 2015, three WASH/SBCC organizations have been working successfully to achieve the above four objectives. In addition to the F & E activities identified, there has been an increase in cross-collaboration between the MOH and WASH partners through the sharing of data and participation in relevant stakeholder technical meetings such as the Uganda National Sanitation Working Group and the Uganda Neglected Tropical Disease Technical Committee. The Uganda Trachoma Control Program provides an excellent example of what can be achieved when different government, public health and WASH sectors collaborate towards a common goal.

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BURDEN OF DISEASE ATTRIBUTED TO WATER-BORNE TRANSMISSION OF SELECTED GASTROINTESTINAL PATHOGENS, AUSTRALIA 2010

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Water is an important source of infectious diseases transmission, however the burden of water-borne disease is not well characterized. We have previously published estimates of the burden of disease caused by *Campylobacter*iosis, non-typhoidal salmonellosis, cryptosporidiosis, giardiasis, and norovirus in Australia in 2010 using number of cases, number of deaths and disability adjusted life years (DALYs). Post-infectious sequelae were included in DALY estimates for *Campylobacter*iosis (irritable bowel syndrome [IBS], reactive arthritis [ReA] and Guillain-Barré syndrome]) and salmonellosis (IBS and ReA). Here we have applied regional WHO estimates of the pathogen-specific proportion of cases attributable to water-borne transmission in 2010 (point estimates and 95% uncertainly intervals [UI]) to our published point estimates of overall disease burden for these pathogens. The WHO estimates for the Western Pacific Region Stratum A (WPR A) included Australia, Brunei, Japan, New Zealand and Singapore. The proportion of cases attributed to water-borne transmission ranged from 0.01 [95%UI 0.00-0.22] for salmonellosis to 0.39 [95%UI 0.03-0.72] for cryptosporidiosis. Norovirus had the greatest number of water-borne cases (479,632 [95%UI 0-1,111,874]) followed by giardiasis (178,275 [95%UI 6,147-418,023]) and *Campylobacter*iosis (85,140 [95%UI 0-247,681]). Deaths were attributed to water-borne transmission of *Campylobacter*iosis (six [95%UI 0-17]), norovirus (four [95%UI 0-9]), and salmonellosis (one [95%UI 0-20]). The water-borne DALY burden was greatest for *Campylobacter*iosis (2,004 [95%UI 0-5,831]), giardiasis (280 [95%UI 10-658]) and norovirus (244 [95%UI 0-566]). Attribution was made at the point of human exposure and disease caused by food contaminated through exposure to dirty water was not attributed to water-borne transmission. Therefore, improvements in water quality has potential to lessen the burden of both water- and food-borne disease. These data can inform Australian guidelines relating to water used for drinking, food production and recreation.

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DETECTING AND ENUMERATING SOIL-TRANSMITTED HELMINTH EGGS IN SOIL: NEW METHOD DEVELOPMENT AND RESULTS FROM FIELD TESTING IN BANGLADESH AND KENYA

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Globally, about 1.5 billion people are infected with at least one species of soil-transmitted helminth (STH). Soil is a critical environmental reservoir of STH, yet there is no standard method for detecting STH eggs in soil. We developed a field method for enumerating STH eggs in soil and tested it in Bangladesh and Kenya. We optimized a method, based on a US EPA method for enumerating Ascaris in biosolids, through a series of recovery efficiency experiments; we seeded soil samples with a known number of *Ascaris suum* eggs and assessed the effect of protocol modifications on egg recovery. We found the recovery efficiency increased when we used 1% 7X as a surfactant compared to 0.1% Tween 80 and two centrifuge flotation steps compared to one. Other protocol modifications, such as sample mixing and sedimentation time, did not impact recovery efficiency. Soil type affected the egg recovery efficiency; sandy samples resulted in higher recovery efficiency compared to loamy samples processed using the same method. We documented a recovery efficiency of 73% for the final optimized method. Soil samples from 100 households in Bangladesh and 100 households in Kenya were processed with the optimized method from June to November 2015. Field staff collected soil samples from the surface layer of soil directly adjacent to the doorway of the house entrance. Both the prevalence and concentration of STH eggs in soil was higher in Bangladesh than in Kenya. In our field tests, we found the prevalence of any STH egg in soil was 78% in Bangladesh and 37% in Kenya. In Bangladesh and Kenya, *Ascaris* was the most common STH (67% and 22%) followed by Trichuris (36% and 21%). We did not detect hookworm eggs in either country, suggesting the method may not be appropriate for hookworm enumeration. The median concentration of STH eggs in soil in positive samples was 0.64 eggs/g dry soil in Bangladesh and 0.15 eggs/g dry soil in Kenya. The proportion of STH eggs determined to be viable was similar in both countries (85.7% in Bangladesh and 83.6% in Kenya). This new method is feasible for detecting STH eggs in soil in low-resource settings and could be a key tool for standardizing soil STH detection globally.

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EVALUATING BEHAVIOR CHANGE IN SINGLE AND COMBINED INTERVENTIONS OF A LARGE-SCALE WATER, SANITATION, HYGIENE AND NUTRITION INTERVENTION TRIAL (WASH BENEFITS), IN RURAL BANGLADESH

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Promoting multiple behavioral interventions together risks limiting sustained adoption of the individual behaviors. WASH Benefits, a large scale efficacy trial, randomly allocated 720 clusters of 5551 pregnant women to a control group, single interventions (water [W], sanitation [S], handwashing [H], nutrition [N]), or combined interventions (WSH and WSHN). Enabling hardware and behavior change was promoted by trained local community health promoters through periodic household visits. In samples of intervention households, we conducted monthly observations beginning 3 months after initiation of the intervention, starting November 2012 to October 2014, to monitor intervention uptake. We analyzed and compared uptake among households receiving single (W, S, H or N) versus combined (WSH & WSHN) interventions. Observed dual pit latrine uptake did not differ significantly among the arms (S: 70%, WSH: 70%, WSHN: 72%; p>0.05). A slightly higher proportion of of households in the single handwashing arm (93%) had water and soap present at the handwashing station near the latrine, compared to the combined intervention arms (WSH: 85%, WSHN: 87%; p<0.01). Detectable residual chlorine in stored water was somewhat higher in households receiving the single water intervention (76%) than combined interventions (WSH: 68%, WSHN: 67%; p<0.05). Among households who received the nutrition intervention, report of feeding lipid-based nutrient supplementation (LNS) to the child (6-24 months) was similar across the arms (N & WSHN: 84%; p>0.05). Rigorous implementation of interventions deployed at large scale achieved high levels of uptake in single and combined intervention arms. However, we found somewhat lower uptake of fully stocking handwashing stations and treating water among households receiving combined interventions compared to households receiving single intervention; uptake differences were small. High uptake of large scale combined WASH and WASHN interventions is possible in the context of an efficacy trial, though further work should assess their effectiveness under programmatic conditions.

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WHO CAN AFFORD GPS POINTS? SCALING A VILLAGE-LEVEL WATER ACCESS INFORMATION SYSTEM IN RURAL ZAMBIA

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An ideal information system for monitoring a country's water system incorporates geocoordinates for all improved water points. However, the generation and maintenance of such an information system is extremely expensive in terms of both finances and human power. Furthermore, a database of geo-located water points alone is insufficient to determine water access of the population – the database must be matched to a population database to determine access. The Ministry of Local Government and Housing (MLGH) leveraged an established mobile-to-web information system of village-level access to sanitation to monitor village-level access to improved water points. The addition of 4 separate data elements allowed for the creation of two indicators: the percent village access of improved water and villages with improved water points that are not functioning. Using District Health Information System (DHIS2), we have seen large disparity in village-level water access within districts and have been able to utilize the information system to direct borehole drilling operations to areas most in need. Furthermore, the approximate geolocation of the water point is being made available through village geo-coordinates. Zambia is now scaling village-level water access monitoring to all rural districts. In addition to demonstrating the power of monitoring water access, we discuss challenges, solutions and opportunities in developing and sustaining nationwide village-level water access monitoring in Zambia.

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QUANTIFYING THE ROLE OF FAILING WATER AND SANITATION INFRASTRUCTURE ON HEALTH, HEALTHCARE COSTS AND SOCIETAL WELLBEING DURING VARYING DISASTER SCENARIOS

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The United Nations Office for Disaster Risk Reduction (UNISDR) estimates that 100 million people are affected by disasters annually, while climate change remains the biggest global health threat of the 21st century. The relationship between climactic variation, environment and zoonotic and vector-borne diseases is well established. A mounting body of evidence further suggests that climate change poses a significant threat to food security, and to microbial, chemical and physical food and water safety. Climate-related food-borne illnesses are predicted to disproportionately affect poor, elderly and young populations, and to be pronounced in low resource settings, where public health infrastructure and human resource capacity is limited or fragile. We created a series of models to estimate the impact of various disaster scenarios that result in varying levels of levels and trends in the quality and scope of key water provision, sanitation and health care delivery infrastructure. A multi-level model was developed encompassing a matrix-map of the interactions between key drivers of water quality, access, provision, and scarcity. Key inputs to health care service quality, accessibility and coverage were used to determine the change in the likely consequences of any breakdowns on these key drivers. This model uses variables from four categories of data; disease incidence, critical infrastructure, climactic variables and food security, to develop estimates of changes in population wide disease burden. These results are then used, along with data on healthcare infrastructure, to estimate marginal impact on healthcare costs, and resulting impact on households, as well as the wider social burden resulting from greater disease burden within the population, such as growth and cognitive development in children and impacts on education, and lifetime earnings. Ultimately the value of this model will be in developing a 'currency' for risk that not just improves our understanding of the true costs to society of various levels of risk of major adverse events, but also allows us to measure the return on investment on early interventions.

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A RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND PILOT STUDY OF SINGLE-DOSE HUMANIZED ANTI-IL5 ANTIBODY (RESLIZUMAB) FOR THE REDUCTION OF EOSINOPHILIA FOLLOWING DIETHYLCARBAMAZINE TREATMENT OF *LOA LOA* INFECTION

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Diethylcarbamazine citrate (DEC) treatment of loiasis is complicated by adverse reactions that are correlated with the number of circulating microfilariae (mf). The cause of these reactions is unknown, but they are accompanied by a dramatic interleukin-5 (IL-5)-dependent increase in eosinophilia and evidence of eosinophil activation. To explore the role of IL-5 driven eosinophilia on post-DEC reactions, 8 adults with parasitologically-confirmed loiasis and <5000 mf/mL blood were enrolled on a randomized, double-blind, placebo-controlled trial of the humanized anti-IL-5 antibody, reslizumab, (1.0 mg/kg IV) administered 3 to 7 days prior to initiation of DEC treatment (9 mg/kg/day for 21 days). Subjects were assessed prior to, at days 1, 2, 3, 5, 7, and 14 and months 1, 3, 6, 12, 18 and 24 post-DEC treatment. The primary endpoint was the reduction in absolute eosinophil count (AEC) during the first week of DEC treatment. Secondary outcomes included the severity of post-treatment adverse events (AE), markers of eosinophil activation, and mf clearance. Baseline characteristics were comparable between the two groups. Single dose reslizumab lowered the AEC by 77% prior to initiation of DEC therapy (vs. a 12% decrease in the placebo group, $p<0.05$). More importantly, reslizumab significantly reduced AEC in the first week of DEC treatment, with peak AEC remaining below baseline in all subjects who received reslizumab and in none of the placebo subjects. Mf clearance occurred within 2 days of initiation of DEC in all 7 mf+ subjects. Mild to moderate AEs were seen in all 8 subjects and were not significantly different between the reslizumab and placebo groups. In summary, although reslizumab was able to block peripheral eosinophilia post-DEC treatment in subjects with loiasis and had no effect on microfilarial clearance, the reduction in AEC appears to have been insufficient to prevent post-treatment AEs. Assessment of eosinophil activation and cytokine profiles is ongoing.

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MOLECULAR DETECTION OF *ONCHOCERCA VOLVULUS* IN SKIN BIOPSIES FROM THE DEMOCRATIC REPUBLIC OF THE CONGO (DRC)

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Defining the optimal diagnostic tools for evaluating onchocerciasis elimination efforts is paramount. The sensitivity of skin skip microscopy decreases as microfiladermia is suppressed, highlighting the need for molecular tools. We assessed the ability of a pan-filarial real-time PCR with melt curve analysis (qPCR-MCA) tool to detect *Onchocerca volvulus* (OV) in residual skin snip biopsies and evaluated the performance of this tool relative to microscopy. Residual skin snip biopsies were collected from 471 people during an onchocerciasis survey in Kisangani, DRC, an area co-endemic for OV, *Mansonella* spp. (MSPP) and *Loa loa* (LL). Melting-temperature (T_m) ranges for species identification were determined using known gDNA. By qPCR-MCA, 43.5% (205) of the samples were negative, 47.5% (224) had OV, 3.8% (18) had MSPP, and 5.1% (24) had LL. An OV specific qPCR (qPCR-O150) was run as validation for all qPCR-MCA(+) samples and 25% of the qPCR-MCA(-) samples. There was 100% concordance for negative samples, and 97% concordance among positive samples: 5 were qPCR-MCA(+) but qPCR-O150(-) while 3 were qPCR-O150(+) but qPCR-MCA(+) for either LL or MSPP but not OV. Sequencing was done for 61 samples because they had a non-OV T_m or a dissociation curve suggestive of mixed infection. Of these, 15 had LL, 30 had *M. perstans* (MP), 6 had OV, and 8 had mixed template chromatograms. Overall, 43.5% of skin snips were negative, 43.7% had OV, 3.0% had LL, 3.2% had MP, and 6.6% had ≥ 1 species. The sensitivity and specificity of microscopy was 79.5% and 95.5% compared to qPCR-MCA and 80.6% and 96% compared to qPCR-O150. Skin snip microscopy was less sensitive than qPCR even in a hyper-endemic setting that received yearly ivermectin. The qPCR-MCA identified 30 cases other filariae that were sequence confirmed. Although this assay detected OV, it was not sufficiently robust to differentiate all species in mixed infections, which had to be resolved by species-specific PCRs. Nevertheless, the qPCR-MCA assay is a useful, rapid screening method able to detect OV and identify samples with mixed infections which will be invaluable for validating other diagnostic assays.

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TOLL-LIKE RECEPTOR 2 EXPRESSION ON IMMUNE CELLS IS ELEVATED IN CURED ASYMPTOMATIC INDIVIDUALS IN LYMPHATIC FILARIASIS

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Various clinical manifestations observed in lymphatic filariasis-endemic (LF-endemic) areas may be partly due to alterations in innate and adaptive immune cell populations expressing toll-like receptor 2 (TLR2) and toll-like receptor 4 (TLR4). The endosymbiotic bacteria *Wolbachia*, have been shown to induce inflammatory responses that are mediated primarily by the toll-like receptors. The use of the mass control drugs, ivermectin and albendazole, and the macrofilaricide doxycycline introduces a new dynamic in the immune response profiles of individuals in LF-endemic areas. In a study conducted in the Western region of Ghana where therapy has been ongoing for a minimum of 5 years, whole blood from a cohort of 428 individuals comprising 15 patent, 64 latent, 98 endemic normals, 101 lymphedema and 150 previously asymptomatic infected but now uninfected ("cleared infection"), were stimulated in 96-well microtiter plates with the TLR-specific ligands PamCSK4, LPS and HKLM. The expression of TLR2 and TLR4 on innate and adaptive immune cells were measured by flow cytometry. Expression of TLR2 was highest on monocytes ($CD14^+$), a dendritic cell sub-population ($CD11c^+$int), a macrophage sub-population ($CD33^+$high) and $CD4^+$ T cells in "cleared infection" individuals. Additionally, significant differences in TLR2 expression were observed between the "cleared infection" and latent individuals ($p < 0.05$) on monocytes; "cleared infection" and lymphedema pathology individuals ($p < 0.0001$) and "cleared infection" and endemic normal individuals ($p < 0.0001$) on $CD11c^+$int; "cleared infection" and lymphedema individuals ($p < 0.0001$ and $p < 0.05$) on $CD33^+$high and $CD4^+$ T cells, respectively. However, no trend or pattern was observed in TLR4 expression on innate and adaptive immune cells in both infected and uninfected individuals, with observed levels being <4% in all cell types examined. Our findings show that therapy leads to heightened recognition of pathogen-associated molecular patterns by TLR2 on innate and adaptive immune cells, and a recovery in immune response in asymptomatically infected LF individuals who have cleared the infection.

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IN VITRO-GENERATED AND *EX VIVO*-ISOLATED HUMAN DENDRITIC CELLS RESPOND SIMILARLY TO LIVE MICROFILARIAE OF *BRUGIA MALAYI*

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Infection by *Brugia malayi*- one of the two major species causing lymphatic filariasis in humans- has been classified by dysregulation of dendritic cells (DC) most associated with microfilarial (mf) antigen exposure. Our previous data have shown that live mf both induce apoptotic cell death and inhibit the mammalian target of rapamycin (mTOR) in human monocyte-derived DC (moDC) generated *in vitro*. The mTOR signaling pathway is an important regulator of cellular metabolism, proliferation, growth, and autophagy. Recent data suggest phenotypic and functional differences exist between *in vitro* generated moDCs and the myeloid DC (mDC) isolated directly *ex vivo* from blood that could lead to a misconstruing of the importance of our *in vitro* findings. Therefore, we sought to compare the responses of these two DC populations to live mf. Elutriated monocytes from healthy volunteers were either cultured with IL-4 and GM-CSF to generate moDCs or sorted *ex vivo* using an antibody cocktail to isolate mDCs ($CD11c^+$/HLA-DR^+/$CD14^-$/$CD16^-$/$CD1c^+$). Once generated, both cell types were exposed to either media alone, live mf, LPS (an mTOR activator), or rapamycin (mTOR inhibitor) for 1 hr. Using immunoblot analysis, we demonstrate that mf, similar to rapamycin, significantly downregulate the phosphorylation of mTOR (and its downstream effectors p70S6K, and 4EBP1 $p<0.05$) in both moDC and mDC. Interestingly, the rapamycin-like effects of mf are also observed when mf are physically separated from DC using transwells. Because cell contact is not required for mf impairment of the mTOR pathway in DC, we suggest that excretory/secretory products from live mf may mediate this effect. The similarity of these two cell types (moDC and mDC) in response to mf extends beyond mTOR inhibition to the induction of apoptotic cell death. When exposed to live mf, both cell types have a marked (~7-fold) increase in cell death as measured by propidium iodine positivity by flow cytometry. Together, these data suggest that while there are phenotypic and functional differences between moDC and mDC, with respect to responses to live mf (and mTOR inhibitors), they appear to function similarly.

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ONCHOCERCA VOLVULUS PROTEOME-WIDE LINEAR EPITOPE SCANNING USING HIGH-DENSITY PEPTIDE MICROARRAYS AND CONFIRMATION OF IMMUNODOMINANT MOTIFS BY ELISA

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We have employed high-density peptide microarrays containing 832,709 partially overlapping 15-mer peptides covering the entire *Onchocerca volvulus* proteome, and screened serum samples of Onchocerciasis patients (n=12) and controls (n=6) for immunoreactivity. After selection of cut-offs for signal intensity and significance, approximately 2000 immunoreactive peptides were identified, of which at least 290 could be considered immunodominant. This pool of immunodominant peptides was further divided into clusters, each cluster characterized by the presence of a motif of 5 or 6 amino acids with invariant anchor points and variability around these anchors. Hence, all peptides belonging to a cluster had at least one such motif. Three of these motifs were strongly represented, e.g. motif 1 was found in 74 peptides, motif 2 in 39 peptides, and motif 3 in 37 peptides. These peptides were found in a large variety of apparently non-related OVOC proteins. For each of these three motifs, we have used at least four peptides for development of a peptide ELISA. Sensitivity and specificity for each peptide was determined using 21 plasma samples from *Onchocerca*-confirmed individuals (FR3 repository), and 187 control plasmas from non-*Onchocerca* endemic regions (healthy, and infections with HIV, HCV, Dengue, *Brugia, Wuchereria* and *Loa*). Within one cluster, the antibody reactivity against e.g. peptide 1 could be inhibited by adding e.g. peptide 2 (from a non-related gene) in solution in ELISA. Sensitivity range for peptides containing motif 1 was between 90.5 % and 100%; and specificity range was between 91.4 % and 94.1 % (results for motif 2 and 3 are pending). The immune response against these peptides was mainly IgG1, IgG3, IgM and IgE. The key amino acids responsible for the immune recognition were determined by micro-array epitope scanning. Taken together, our data demonstrate that part of the humoral immune response induced by infection with *O. volvulus* is directed against a small number of highly abundant peptide motifs present in apparently structurally nor functionally related OVOC genes.

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THE EFFECT OF *LITOMOSOIDES SIGMODONTIS* INFECTION ON IGE-MEDIATED ANAPHYLAXIS IN SENSITIZED MICE

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IgE-mediated anaphylaxis is a life-threatening condition. Binding of IgE with its cognate antigen causes basophils and mast cells to rapidly release pre-formed inflammatory mediators. While numerous animal studies have reported that helminths can prevent allergic sensitization, only 4 studies have evaluated the effects of infection on pre-existing allergy. Since chronic helminth infection suppresses basophil and mast cell function, we hypothesized that chronic infection would protect against the clinical symptoms of anaphylaxis in previously sensitized mice. Mice were sensitized by weekly intraperitoneal (IP) injection of either ovalbumin (OVA)/alum or PBS/alum for 3 weeks. At 5 weeks, mice were infected with *Litomosoides sigmodontis* (*L.s.*), a rodent filarial parasite, or mock-infected. Ten weeks post-infection, immunological and clinical parameters were measured before and after IP challenge with OVA. In sensitized mice, chronic *L.s.* infection resulted in serum levels of OVA-specific IgE that were an average of 50% lower than those observed for mock-infected mice (6,296 vs. 13,056 pg/mL, p-value- 0.1494). Chronic infection also caused a modest reduction in OVA-specific IgG2a levels. Following challenge, serum levels of murine mast cell protease 1 were significantly lower in sensitized mice that were *L.s.*-infected as compared to mock infected (86,276.81 vs. 276,635.2 pg/mL, p- value= 0.0385). With respect to clinical symptoms, *L.s.*-infected mice that had been previously sensitized exhibited an average drop in core body temperature of 4.3 °C after 1 hour. Although less than the average 6.3°C drop observed in sensitized and mock-infected mice, the difference was not statistically significant. These results suggest that chronic helminth infection reduces allergen-specific IgE levels and allergen-driven mast cell degranulation in previously sensitized animals.

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MIXTURE MODELING TO DETERMINE POPULATION-SPECIFIC CUTOFFS FOR IMMUNOLOGIC ASSAYS IN NEGLECTED TROPICAL DISEASE SETTINGS APPROACHING ELIMINATION

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As Neglected Tropical Disease (NTD) programs succeed and transmission intensity declines, the ability to discriminate between positive and negative antibody tests becomes increasingly challenging. Previous techniques for defining diagnostic test cutoffs are no longer sufficient as prevalence rates decline towards zero. With varying degrees of non-specific background reactivity across populations and the absence of a gold standard, an objective yet flexible approach for cutoff determination is needed. Mixture modeling allows for the probabilistic representation of subpopulations within an overall population. By fitting a mixture model to continuous data, members of the overall population can be assigned to groups (e.g., positive and negative), and the uncertainty of that classification can be calculated based on the associated conditional probabilities. These groups can be used to create an absolute cutoff and a pre-specified indeterminate range (e.g. greater than 5% uncertainty), resulting in positive, negative and indeterminate classifications. The number of groups may be specified in advance or optimized by an algorithm using model selection criteria, such as the Bayesian Information Criterion. We performed mixture modeling on standardized ELISA results from two post-treatment NTD settings. Antibody responses to a lymphatic filariasis recombinant antigen (Wb123) were analyzed via normal mixture modeling utilizing a two-group model which yielded a cutoff of 0.092 and an indeterminate range of (0.082, 0.100). This corresponded to 96.1% negative, 2.3% indeterminate and 1.5% positive results. The same methods were used to analyze responses to a recombinant onchocerciasis antigen (Ov-16), resulting in a cutoff of 0.63 (0.57, 0.68) with 96.7% negative, 1.5% indeterminate and 1.8% positive results. These results demonstrate the utility of mixture modeling as a tool to provide population-specific diagnostic cutoffs with a corresponding indeterminate group that reflects our certainty regarding the cutoff. Such an approach may benefit other neglected and infectious disease programs driving towards elimination.

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THE LIVE ATTENUATED CHIMERIC VACCINE RWN/DEN4Δ30 IS WELL-TOLERATED AND IMMUNOGENIC IN HEALTHY FLAVIVIRUS-NAÏVE ADULT VOLUNTEERS 50-65 YEARS OF AGE

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West Nile Virus (WNV) is the leading vector-borne cause of meningoencephalitis in the U.S. Many infections are asymptomatic. Severe illness is most common in the elderly, including hepatitis and meningitis. Complications include: paralysis, coma, and death. The second largest outbreak of WNV in the U.S. occurred in 2012 (5,674 cases, including 2,873 cases of severe neurologic disease, 286 deaths). The NIH candidate vaccine is a recombinant live attenuated (LA) WNV vaccine based on the chimerization of wild type WNV (NY99 genome) with the LA dengue virus serotype 4 vaccine rDEN4Δ30. Previous vaccine studies were conducted in healthy volunteers at 103, 104 and 105. Based on the safety and immunogenicity data of previous studies, a dose of 104 was chosen. A randomized placebo controlled phase I trial was performed. 28 healthy flavivirus-naïve volunteers aged 50-65 were enrolled and randomized to receive 104 PFU of rWN/DEN4Δ30 or placebo at 0 and 6 months. Neutralizing antibody (Nab) to WNV was measured at day 28, 56, 90, and 180 following first vaccination and days 208, and 236 following second vaccination. Nab was measured to PRNT50 against WT WNV conducted in a BSL3 lab at LID. Seroconversion was defined as > 4 fold rise in Nab titer to the wild-type WNV parent virus at study day 90 post first vaccination compared with day 0. Following first dose, 3 subjects had detectable viremia with a maximum peak titer of 0.7log10 PFU/mL. No subject was viremic following second dose. 95% of vaccines seroconverted following first dose. The Geometric mean peak titer (GMT) at day 90 was 65.3, D180 was 27.63 and D360 was 34.52. Main AEs reported were similar in placebos: headache (25% vaccinees (V), 12.5% placebo (P)), Fatigue 10% (V), 37% (P), and nausea 25% (V), 12.5 (P). rWN/DEN4Δ30 was found to be well tolerated and immunogenic in adults aged 50-65. Vaccination induced a 95% seroconversion rate at day #90 after a single dose. As WN is more severe in patients over 50, these results underscore the potential use for rWN/DEN4Δ30 vaccine in this population, given the unpredictable and sporadic outbreaks of WNV.

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ACUTE AND DELAYED MORTALITY FOLLOWING WEST NILE VIRUS INFECTION IN TEXAS

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West Nile virus (WNV) has a well characterized acute disease process; however, the long term survival and contributors of death at a population level are not well understood. In this study, we sought to investigate all-cause and cause-specific mortality following WNV infection.Our study

population consisted of 4144 Texas residents who were infected with WNV from 2002-2012. Out of this population, we identified 554 deaths (13%). Our analysis focused on both acute deaths (n=286) occurring < 90 days after infection, and delayed deaths (n=268) occurring among those who survived the first 90 days. Standardized mortality ratios (SMRs) adjusted for age, sex, and calendar year were calculated according to ICD10 chapters. We found a substantial number of reported WNV cases died within the first 90 days of infection (286 out of 4144; 7%), primarily due to their WNV illness or an unspecified infectious origin. Delayed mortality occurred in 10% (210/2113) of initially surviving patients diagnosed with West Nile Neuroinvasive disease (WNND) and in 3% (58/1742) of those diagnosed with West Nile Fever (WNF). WNND cases experienced increased risk of delayed death due to infectious (SMR: 4.7, 95%CI: 3.2-6.9) and renal causes (2.6, 1.4-4.7). We also found increased risk for all-cause mortality in those who were under 60 years of age at the time of infection (SMR 1.98, 95% CI: 1.5-2.6) but not among those over 60 years (0.99, 0.8-1.2). Cases under 60 years exhibited increased risk of delayed mortality due to renal (11.4, 4.7-27.3), infectious (5.3, 2.5-11.2), digestive (3.9, 1.9-8.1), and circulatory (2.0, 1.2-3.4) causes. We present the first population-level evidence of acute and delayed mortality among a considerable proportion of patients with a history of WNV infection. Our data provide further evidence to the literature supporting continued morbidity and mortality years after WNV infection.

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A SINGLE MUTATION IN THE ENVELOPE PROTEIN ALTERS FLAVIVIRUS ANTIGENICITY, STABILITY AND PATHOGENESIS

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Flaviviruses include clinically significant pathogens such as West Nile (WNV), dengue (DENV), and Zika (ZIKV) viruses. Flaviviruses particles are covered with a dense array of three-domain (DI, DII, DIII) envelope (E) proteins, which are targeted by neutralizing antibodies following infection or vaccination. The structural flexibility or 'breathing' of flavivirus envelope (E) proteins allows virions to sample an ensemble of conformations at equilibrium. The molecular basis and functional consequences of virus conformational dynamics are poorly understood. By analyzing a large panel of WNV E variants, we identified mutations capable of changing the structural ensemble sampled by virions at equilibrium. Here, we describe the antigenic and biological consequences of a mutation at residue 198 (T198F) of the E DI-DII hinge. T198F displayed increased sensitivity to neutralization a monoclonal antibody targeting a poorly exposed epitope in the DII fusion loop. Increased exposure of this cryptic epitope was accompanied by changes in virus stability; following prolonged incubation in solution at physiological temperatures, the T198F mutation resulted in a 3-fold reduction in the half-life of infectious WNV. Introduction of a mutation at the analogous residue of the dengue virus E protein similarly increased accessibility of the fusion loop epitope and decreased virus stability in solution, suggesting that this residue modulates the structural ensemble sampled by distinct flaviviruses at equilibrium. Despite resulting in a 3-fold reduction in the stability of WNV in solution, the T198F mutation did not substantially impair *in vitro* replication kinetics. However, *in vivo* studies in mice revealed that the T198F mutation attenuates lethal WNV infection in a B-cell dependent manner. Overall, our study provides insight into the molecular basis and the *in vitro* and *in vivo* consequences of flavivirus breathing that has the potential to inform the design of effective vaccines and therapeutic agents.

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AN OPTIMIZED SYNTHETIC TLR-4 AGONIST ADJUVANT FORMULATION INDUCES DURABLE AND FUNCTIONAL IMMUNITY WHEN COMBINED WITH A CLINICAL-STAGE RECOMBINANT WEST NILE VIRUS VACCINE ANTIGEN

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West Nile virus (WNV) is a mosquito-transmitted member of the Flaviviridae family that has emerged in the 21st century to become a public health threat. Given the sporadic nature of WNV epidemics both temporally and geographically, there is an urgent need for a vaccine that can rapidly provide effective immunity. Protection from WNV infection is correlated with antibodies to the viral envelope (E) protein, which encodes receptor binding and fusion functions. Despite many promising E-protein vaccine candidates, there are currently none licensed for use in humans. This study reports the optimization of a WNV vaccine candidate containing a clinical-stage WNV recombinant E-protein antigen (WN-80E) and a TLR-4 agonist adjuvant containing a synthetic Lipid A TLR-4 agonist (SLA) and the saponin QS21 (SLA-LSQ). We have optimized adjuvant components for rapid induction of potent antiviral immunity in murine models, and find that both SLA and QS21 individually stimulate the production of multi-functional Th1 CD4+ T-cells (IFNγ+/TNFα+/IL-2+), as well as an increase in the number of germinal center B-Cells (CD95+/GL7+) in a dose dependent manner. Consistent with induction of Th1 biased cellular immunity, the humoral response following adjuvanted immunization in mice is focused toward production of class-switched IgG2c antibodies, resulting in high levels of virus neutralization activity. Importantly, we observe significantly increased neutralizing titers in mice given formulations which contain both SLA and QS21 compared to either component alone. Using an optimized vaccine formulation, we demonstrate induction of durable immunity (300 days) following a single immunization in mice, and stimulation of functional protective immunity in a Syrian hamster challenge model of WNV disease. Taken together, these studies demonstrate the utility of SLA-LSQ adjuvant formulations for induction of functional and durable West Nile Virus immunity.

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DIFFERENTIAL MECHANISMS OF WEST NILE VIRUS-INDUCED PATHOGENESIS IN BIRDS

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West Nile virus (WNV) is maintained in North America in an enzootic cycle between highly susceptible passerine birds and mosquitoes. However, the mortality rates and viremia profiles of birds in response to WNV infection vary dramatically between viral strains and avian species. For example, American crows (AMCRs) inoculated with the NY99 strain of WNV manifest high viremias with concomitantly high mortality rates. However, AMCRs inoculated with a strain of WNV containing a pro-to-thr mutation at amino acid 249 of NS3 (NS3-249T) exhibit significantly lower viremias and mortality rates. These viremia profiles are inversely correlated with interferon (IFN)-α expression. In contrast, we found that the viremia and mortality rate of the NS3-249T virus is higher than that of the WT NS3-249P virus in 2-day-old chicks. In order to better understand these viral- and host-specific differences in WNV replication, a chicken fibroblast

cell line was infected with NS3-249T or 249P viruses, with and without chicken IFN-α pre-treatment. While both viruses were sensitive to IFN-α treatment, the NS3-249T virus replicated to a higher titer, suggesting the increase in chick viremia *in vivo* may be due to increased intracellular replication, rather than a differential innate immune response. To identify the cell populations contributing to the replication of WNV in chicks, 2 day-old chicks were inoculated with a WNV mutant restricted for leukocyte replication through the insertion of multiple leukocyte-specific miRNA target sequences into the 3' UTR. Previous studies have shown that a leukocyte-restricted WNV mutant replicates poorly in AMCRs, suggesting leukocytes are important sites of replication. Unexpectedly, the leukocyte-restricted virus exhibited increased viremia and mortality in chicks. This suggests that chick and AMCR leukocytes play very different roles during *in vivo* WNV infection as critical sites of viral replication or effectors of innate immune responses, respectively. Further studies of infected birds, including sequencing of the avian transcriptome, will help to define the avian innate immune response to WNV infection.

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THE EFFECT OF CO-INFECTION WITH DENGUE, CHIKUNGUNYA AND ZIKA VIRUS ON VECTOR COMPETENCE OF *AEDES* MOSQUITOES

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With the recent emergence of both Chikungunya virus (CHIKV) and Zika virus (ZIKV) in the Americas, there are now three arboviruses cocirculating in large areas of South and Central America that are all transmitted by *Aedes* mosquitoes: dengue virus (DENV), CHIKV and ZIKV. Clinical signs can be similar and while cases of coinfections have been reported to occur, the incidence rate of these is unknown and may be underestimated due to the lack of virus specific diagnostic tools. Coinfected viremic patients could expose feeding mosquitoes to multiple arboviruses. Interestingly, in 2015 an increase in CHIKV infections coincided with a drop in dengue cases in Mexico and Colombia. While this could be due to yearly variation, it could also be related to the introduction of CHIKV which may be outcompeting DENV in mosquitoes. However, the impact of coinfection on the ability of relevant mosquitoes to transmit any of these viruses (i.e. their vector competence) has not been determined. Therefore, we determined the competence of *Ae. aegypti* (Poza Rica, Mexico) and *Aedes albopictus* (Florida) mosquitoes exposed to bloodmeals containing more than one *Aedes*-borne arbovirus. Specifically, mosquitoes were given a blood meal containing American strains of DENV-2, CHIKV or ZIKV in single infections, as well as combinations of the three viruses as double and triple infections. Mosquitoes were kept for 5, 7, 9 and 14 days extrinsic incubation, at which point mosquito bodies, legs and saliva were collected to determine infection, dissemination and transmission rates. Presence of viral RNA was determined by multiplex qRT-PCR for DENV-2, CHIKV and ZIKV in order to determine RNA levels for the individual viruses in mosquitoes exposed to more than one virus. Preliminary results suggest that coinfection may impact vector competence in a virus-specific manner.

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THE INFLUENCE OF GENETIC BOTTLENECKS, RNA INTERFERENCE-MEDIATED DIVERSIFICATION AND SELECTIVE CONSTRAINT ON THE EVOLUTION OF A TICK-BORNE VIRUS

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Within hosts, flaviviruses exist as a heterogeneous population of closely related viral variants termed a mutant swarm. While considerable attention has been directed toward studying the population dynamics of mosquito-borne flaviviruses, little is known about tick-borne flavivirus populations. This is disconcerting due the increased public health relevance of Powassan virus (POWV), the sole North American member of the tick-borne encephalitis complex. Therefore, we assessed the population complexity of POWV lineage II (deer tick virus; DTV) in both a vertebrate host and invertebrate vector and examined the influence of RNAi targeting on DTV populations. 11-week old mice were intra-peritoneally infected with DTV and subsequently fed upon by Ix. scapularis larvae and nymphs. Infected ticks were collected at each life stage through adulthood and RNA-Seq and sRNA libraries were prepared, sequenced and analyzed. We found that DTV populations were subject to a strong bottleneck once introduced into mice, but then rapidly diversified. Subsequently, the populations experienced another severe bottleneck during horizontal transmission to ticks. During transstadial transmission, however, diversification was constrained by strong purifying selection. Despite the relatively limited diversity observed in ticks, we found that RNAi targeting intensity was significantly positively correlated with the presence of intrahost single nucleotide variants (iSNVs); findings similar to what has previously been described for West Nile virus (WNV) in mosquitoes. While the overall DTV population dynamics greatly differ from those of WNV, these findings provide experimental evidence consistent with DTV ecology and support their slow long term evolutionary trends. Together, these data highlight that selective pressures incurred at a molecular level, such as RNAi, may be similar for most if not all arthropod-borne flaviviruses, but that differences in viral ecology can greatly influence the population dynamics of individual flavivirus members.

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MOSQUITO MIDGUT FREP1 IS A POTENTIAL UNIVERSAL MALARIA TRANSMISSION-BLOCKING VACCINE

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Malaria remains a devastating disease. Transmission-blocking vaccines (TBVs) have been recently considered as a promising approach for the elimination and eradication of malaria. We recently discovered a mosquito midgut protein FREP1 that facilitates parasite transmission through direct binding to parasites and it is easily accessible to antibodies co-ingested with blood. Here, we demonstrated that anti-FREP1 antibodies blocked transmission of multiple *Plasmodium* species (*Plasmodium berghei*, *Plasmodium vivax,* and *P. falciparum*) to multiple *Anopheles* species (*A. gambiae* and *A. dirus*). Sequence comparison of FREP1 orthologs found that a fibrinogen-like (FBG) domain is highly conserved (>90% identity) among *Anopheles* species from different continents, while the sequence similarity between FBG and human fibrinogens is only about 10%. Immunization of mice with purified recombinant FREP1 shows no significant difference of alanine aminotransferase activity between anti-FREP1 serum and the pre-immune serum. Moreover, the anti-FREP1 serum did not show any cross-reactions with human fibrinogens. Thus, FREP1 is non-toxic or unlikely to cause autoimmune response in mammals. Notably, mice immunized with purified FBG effectively blocked *P. berghei* transmission (82.1% and 70.1% blockade) to *An. gambiae* in two independent bioassays respectively. Anti-FREP1 serum from the immunized mice also blocked over 90% infection of *P. falciparum* in standard membrane-feeding assays (SMFA). In summary, our data support that FREP1 is a promising universal TBV antigen to block the transmission of multiple *Plasmodium* species to multiple *Anopheles* species.

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SKIN SCARIFICATION WITH *PLASMODIUM FALCIPARUM* CS PEPTIDE VACCINES USING SYNTHETIC TLR AGONIST ADJUVANTS ELICITS CHEMOKINE/CYTOKINE PATTERNS THAT CORRELATE WITH INDUCTION OF SPOROZOITE NEUTRALIZING ANTIBODIES

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Sterile immunity can be elicited by immunization with sporozoites, however, the production, storage and administration of whole parasite vaccines present numerous logistical hurdles. Vaccination by skin scarification (SS), as used for mass immunization during the Smallpox Eradication Programme, may more closely mimic the natural route of sporozoite inoculation by mosquito bite. We investigated SS immunization using synthetic peptides containing minimal T and B cell epitopes of *Plasmodium falciparum* CS protein combined with TLR agonists as adjuvants. In a murine model, SS immunization with CS peptide in the oil emulsion AddaVax containing TLR-7/8 and -9 agonists, but not AddaVax without TLR agonists, elicited high levels of systemic sporozoite neutralizing antibody, Th1- type CD4+ T cells and resistance to challenge by bites of mosquitoes infected with transgenic rodent parasites expressing *P. falciparum* CS repeats. Immunogenicity of SS delivered vaccine was demonstrated with either branched or linear peptide containing minimal T and B cell epitopes, indicating that induction of sporozoite neutralizing antibody was not dependent on antigen form. Standard serological assays for measuring the magnitude, fine specificity or affinity of anti-repeat antibodies were not predictive of the differences in levels of sporozoite neutralizing antibodies detected in functional assays based on transgenic parasites. To determine the immunological mechanisms relevant to the initiation of the enhanced levels of sporozoite neutralizing antibodies, we examined a panel of cytokines and chemokines elicited by SS using various TLR agonists alone and in combination. Cellular sources of these cytokines/chemokines were examined by immunohistology and flow cytometry using fluorescent reporter cells including Langerhan cells and dendritic cells. Patterns of chemokines/cytokines were detected in serum at early time points that correlated with enhanced immunogenicity of SS delivered vaccines.

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HUMORAL IMMUNE RESPONSES TO AN ADJUVANTED SELF-ASSEMBLING PROTEIN NANOPARTICLE (SAPN) MALARIA VACCINE DISPLAYING THE NANP REPEAT AND αTSR REGIONS OF THE *PLASMODIUM FALCIPARUM* CIRCUMSPOROZOITE PROTEIN

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The Circumsporozoite Protein (CSP) is the predominant protein on the surface of the sporozoite and the most thoroughly studied pre-erythrocytic vaccine candidate. It is composed of an N-terminal domain, a conserved pentapeptide protease cleavage site termed region I, a tetra peptide NANP repeat region, a short conserved sequence termed region III, and a C-terminal region with sequence homology to the thrombospondin type-1 repeat superfamily (TSR). We have previously reported that a self-assembling protein nanoparticle, PfCSP-KMY-SAPN, presenting NANP repeats and three human HLA epitopes of the CSP induced very strong protective immune responses in mice. However, when tested in NHP, immune responses were low even when an adjuvant was used. To improve the vaccine induced immune response we have produced FMP014, a SAPN displaying NANP repeats and the entire αTSR domain of CSP. The αTSR in the SAPN uses two Cys-Cys disulfide bonds to stabilize the hydrophobic pocket and core link found in the native protein. Here we report the analysis of the humoral immune responses of C57Bl/6 mice to FMP014 combined with three different Army Liposomal Formulations containing monophosphoryl lipid A with or without QS-21, and Alhydrogel® (ALFA, ALFQ and ALFQA). Intramuscular injection of C57Bl/6 mice with each FMP014/ALF formulation induced high titer anti-NANP repeat, anti-αTSR domain, and cytophilic IgG2b antibodies with high avidity. However, FMP014 adjuvanted with ALF containing QS-21 (ALFQ) induced significantly higher anti-CSP specific antibodies to the NANP repeats and the αTSR domain compared to those without QS-21. Antibodies to both the NANP repeat region and the αTSR domain conferred protection to mice against challenge from an otherwise lethal dose of a transgenic *P. berghei* parasite expressing the full-length *Plasmodium falciparum* CSP.

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IMMUNOMECHANISM OF PROTECTION FOR E140, A PRE-ERYTHROCYTIC VACCINE CANDIDATE

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A malaria vaccine to prevent infection is greatly needed and essential for a comprehensive malaria eradication program. Pre-erythrocytic (PE) antigens are capable of inducing an immune response resulting in sterile protection, as shown by the RTS,S vaccine. *Plasmodium falciparum* PE antigens are also targeted by efficacious whole sporozoite vaccines and could be the basis of a subunit vaccine. We have identified a single PE *P. yoelii* antigen (E140) capable of sterilely protecting CD1 mice in the range of 71% to 100%, alone and in combination with other antigens, respectively. Initial examination of E140-specific immune responses showed significant CD4+ and CD8+ T cell responses upon DNA-prime/Ad5-boost immunization. We also observed an antibody response that statistically correlated with sterile protection. We have further characterized the T cell responses to E140 immunization evaluating the function of CD4+ and CD8+ T cells, including IFN-gamma, TNF, IL-2, and MIP1alpha. In vivo T cell depletion experiments in mice are being conducted to ascertain the requirement of these cell types for protection. We are also conducting concomitant antibody transfer studies in mice to establish the role of anti-E140 antibodies to protection. These promising data are evidence of the potential of E140 and support further development of the *P. falciparum* E140 ortholog in a subunit malaria vaccine.

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DEVELOPMENT OF A SEMI-SYNTHETIC WHOLE PARASITE VACCINE

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We are developing whole parasite vaccines to protect against the blood stages of malaria and showed that chemically attenuated vaccines can protect against rodent malaria parasites. This approach is being translated to the clinic. However, issues relating to production, storage and delivery present obstacles that impede development of this vaccine and other whole parasite approaches. We are therefore developing a synthetic vaccine delivery system in which killed blood stage parasites are encapsulated within liposomes that are targeted to dendritic cells using mannosylated lipid core peptides (MLCPs). MLCP-liposomes were taken-up efficiently by antigen presenting cells which then upregulated expression of MHC-II and co-stimulatory molecules, CD80 and CD86. Immunization

of mice with MLCP-liposome vaccine formulations, without adjuvant, generated enhanced levels of activated T cells in peripheral blood and vaccinated mice were completely protected from challenge infection with different species of rodent malaria. Liposome formulations are highly promising delivery systems for a human malaria vaccine.

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CORD BLOOD ANTI-PFSEA-1 AND PROTECTION FROM SEVERE MALARIA IN INFANTS

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Plasmodium falciparum malaria is a leading cause of morbidity and mortality in developing countries, infecting hundreds of millions of individuals and killing up to one million children in sub-Saharan Africa each year 1,2. In holoendemic areas, children suffer the most from malaria, particularly after six months of age. Both the relative resistance to infection and severe malarial disease (SM) expressed by neonates and young infants, as well as the hypothesis that this resistance is mediated by maternally derived IgG has been recognized for decades 3-5. Despite these early observations, the targets of protective, maternally derived cord blood antibodies remain elusive. Recently, we demonstrated that antibodies to Pf Schizont Egress Antigen-1 (PfSEA-1) predict decreased risk of SM in 1.5-4 yr olds living in a holoendemic area of Tanzania 6. Here we demonstrate, in the same cohort, that maternally derived anti-PfSEA-1 antibodies cross the placenta, are detectable in cord blood, and cord blood levels of these antibodies predict significantly decreased risk of SM in infants for up to 12 months after birth. Further, in maternal vaccination studies in mice, pups born to dams that were immunized with PbSEA-1 prior to pregnancy had significantly lower parasitemia and longer survival times following lethal *P. berghei* ANKA challenge compared to pups born to dams treated with adjuvant alone. Together, these results identify, for the first time, a parasite specific target of maternal antibodies that transfer to the fetus and are associated with protection from SM and suggest that vaccination of pregnant women with PfSEA-1 may afford a survival advantage to their offspring.

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IMMUNOGENICITY OF CHAD63/MVA ME-TRAP MALARIA VECTORED VACCINE IS NOT AFFECTED BY CO-ADMINISTRATION WITH ROUTINE EPI VACCINES IN A RANDOMIZED CONTROLLED TRIAL IN GAMBIAN INFANTS AND NEONATES

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Recent global estimates show that *Plasmodium falciparum* malaria remains a major public health concern. An effective vaccine could complement existing control measures. Heterologous prime-boost vaccinations using chimpanzee adenovirus 63 (ChAd63) and modified vaccinia Ankara (MVA) encoding ME-TRAP have consistently shown acceptable safety, excellent immunogenicity and substantial efficacy in African adult and paediatric populations. If licensed, this vaccine will be given to infants, who receive routine childhood immunizations. Here, we evaluate the immunogenicity and possible interference of ChAd63/MVA ME-TRAP when co-administered with routine Expanded Programme Immunization (EPI) vaccines in young infants. We enrolled 65 Gambian infants and neonates into 3 groups aged either 16, 8 or 1 week old at first vaccination and randomized them to receive either ME-TRAP vaccine or control. All participants received EPI vaccines according to the national programme. Safety was assessed by the description of vaccines-related adverse events including clinical assessments, biochemical and haematological tests. Immunogenicity was evaluated using anti-TRAP IgG ELISA, interferon-gamma ELISPOT and flow cytometry. Serology was performed to confirm all infants achieved protective titres to EPI vaccines. The vaccines were well tolerated in all age groups with no vaccine-related serious adverse events. High-level TRAP specific IgG and T cell responses were generated after boosting with MVA. Particularly, $CD8^+$ T cell responses, previously found to correlate with protection, were induced in all groups. Antibody responses to EPI vaccines remained at protective levels. While difficult to induce in neonates with protein or polysaccharide vaccines, potent humoral and cellular immunity were generated by heterologous prime-boost immunization with ChAd63/MVA ME-TRAP in young infants and neonates. Co-administration of routine EPI vaccines did not interfere with these responses. The EPI vaccines also retained protective antibody titres following administration of the malaria vaccines, supporting further evaluation of this regimen in infants.

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FIVE-YEAR OUTCOMES OF A RANDOMIZED TRIAL OF SCHOOL VS. COMMUNITY-BASED MASS DRUG ADMINISTRATION FOR *SCHISTOSOMA MANSONI* CONTROL IN WESTERN KENYA

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Current WHO mass drug administration (MDA) recommendations for schistosomiasis control are based on prevalence in school aged children. At the time they were developed, the guidelines were contingent upon two hypotheses: 9-12 year old children are a reliable indicator of the overall level of infection in the population, and once annual MDA will be sufficient to reduce infection levels. Further, the guidelines were originally developed for morbidity control and may no longer be adequate for the WHA 54.19 and 65.21 resolution targets which now includes elimination of schistosomiasis. We conducted a large trial involving 150 communities in western Kenya as part of the Schistosomiasis Consortium for Operational Research and Evaluation (SCORE) to compare different MDA distribution approaches and frequency of delivery on *Schistosoma mansoni* infection prevalence and intensity. For 4 years, community wide (CWT) or school based treatment (SBT) was provided annually, every other year or only the first 2 years to 25 randomly assigned communities in each of 6 study arms. In the fifth year, communities in all arms were evaluated. At the end of the 5 years, communities that had received a total of 4 treatments had lower prevalence ($P = 0.0041$) and intensity ($p = 0.0024$) than those that had received 2 treatments. However, calculating impact relative to cost suggests that even though 2 treatments reduced prevalence less than 4 treatments, the 2 treatment approach may be more cost effective. We evaluated first year pupils in years 1, 3 and 5 as a proxy measure of the force of transmission and showed that both CWT and SBT approaches appeared to impact community transmission levels. Despite the general reduction in prevalence and intensities of *S. mansoni* infections, 44% of the 50 communities that received annual treatment,

either by CWT or SBT, had < 20% change in prevalence over the five year period, indicating that MDA alone may not be sufficient to achieve control or elimination.

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FEMALE GENITAL SCHISTOSOMIASIS IN ABEOKUTA, NIGERIA

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Female Genital Schistosomiasis (FGS) is an emerging public health problem for young girls and women of childbearing age living in urogenital schistosomiasis endemic areas. The disease is associated with vaginal itching and discharge, post-coital bleeding, genitopelvic discomfort, marital discord, infertility, preterm labour, anaemia, menstrual disorders, and painful sexual intercourse. Over 44 million women living in sub-Saharan Africa (SSA) are currently affected by FGS. However, this disease has received little or no attention in Nigeria, which is the most endemic country in sub-Saharan Africa. A study was conducted in four *Schistosoma haematobium* endemic communities of Abule-titun, Imala-Odo, Apojola and Ibaro in Abeokuta to investigate the occurrence of FGS and its associated risk factors in young girls and women of childbearing age (age range 5-49 years). A total of 317 females were examined, of which 150 (47.3%) of them had haematuria (blood in urine), and 149 (47.0%) had pre-patent ova of *Schistosoma haematobium* in their urine respectively. There was significantly ($p < 0.05$) higher prevalence (121, 64.7%) and intensity of infection (1.0659±0.1251) in younger girls (aged 5-15 years) than their older counterparts. Using the standardised virgina discharge colour chart, 4(1.3%) cases of FGS were identified. Full gynaecological examination of 20 participants confirmed 14 (70.0%) cases of FGS. Gynaecological abnormalities included 10 (71.4%) of the females with grainy-sandy patches, 6 (42.9%) with yellow sandy patches, 1 (7.1%) with nabothian cysts and rubbery papules in their vaginal and cervical wall. Bathing (92.7%), fetching (52.4%), fishing (93.4%) and washing clothes (96.5%) at the local dam were reported risk factors associated with *S. haematobium* infection. This study has confirmed that the presence of FGS in Abeokuta, Nigeria. Its impacts and implications on the reproductive health of young girls and women of childbearing age living in urogenital schistosomiasis endemic communities are yet to be documented.

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PROGRESS TOWARDS SCHISTOSOMIASIS CONTROL AND ELIMINATION FROM 2004 TO 2015 IN 28 HEALTH DISTRICTS IN MALI

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Mali has implemented the national schistosomiasis control program since 2005, supported initially by Schistosomiasis Control Initiative and then by Helen Keller International with funding from the USAID's NTD projects managed by RTI International. The main activities include mass drug administration (MDA) and impact evaluations. During 2014-15, 28 health districts (HDs) in Segou, Sikasso, Kayes, Mopti and Koulikoro regions were evaluated. Two or three sites in each HD with 50-60 school-age children at each site, for a total of 59 sentinel sites and 3575 children assessed. All HDs had received six or seven rounds of MDA, except two untreated. The aim of the studies was to evaluate the current status of schistosomiasis and adjust the MDA strategies in each district. The data from 2014-15 were compared to data from the baseline in 2004-05 to understand how prevalence has decreased over time. At baseline, the median prevalence of infection was 49.7% (range 0.29% to 98.5%) and the median prevalence of heavy infection was 12.6 % (0.3% to 78.9%). During the 2014-15 evaluation, the median prevalence of infection was 13.2% (range 0.6% to 83.6%) and the median prevalence of heavy infection was 3.4% (range 0.03% to 25.8%). Thus the median prevalence decreased greatly following the rounds of MDA. As a result ten out of 28 HDs (35.7%) will change their MDA cycle and targets for 2017. Eight out of 28 (28.6%) have achieved schistosomiasis morbidity control, meaning less than 5% of heavy infections in the sentinel population and another eight (28.6%) HDs had achieved the criteria for schistosomiasis elimination, meaning less than 1% of heavy infections in sentinel populations. To move towards the control and elimination goals, additional activities have been identified: snail control, sanitation improvements of river banks and health education. Despite the success, some questions remain, for example, understanding the reasons for persistence of elevated prevalence in three HDs. The evaluation provided an opportunity for the national program to accelerate progress towards elimination and/or control in districts with differing epidemiological profiles.

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COMMUNITY DIALOGUES FOR PREVENTION AND CONTROL OF SCHISTOSOMIASIS IN MOZQAMBIQUE

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A prerequisite for successful schistosomiasis prevention and control is that affected communities have an understanding of the disease. Malaria Consortium conducted a study in four districts of Nampula province to explore whether the community dialogues approach is effective in improving schistosomiasis prevention and control at community level. The approach prompts communities to select volunteers who subsequently receive training and conduct regular dialogues. This allows communities to explore how they are affected, identify locally relevant solutions and plan for taking communal action. For this study, 157 volunteers were trained and equipped with a visual toolkit. Two six-month cycles of community dialogues were conducted. The study used a mixed-methods design: i) representative household surveys of knowledge, attitudes and practices (KAP) at baseline (n=791) and endline (n=795), ii) 25 focus group discussions with volunteers and community members and four in-depth interviews with district health officials, iii) analysis of monitoring and evaluation forms (n=1,462) and observation reports (n=11), and iv) analysis of stories of change written up by community dialogue participants (n=51). At baseline, correct knowledge of how schistosomiasis is transmitted or prevented was low. KAP indicators improved over the lifetime of the project. At baseline 18% could name at least one risk behaviour correctly. At endline, this had increased to 30%. Community participation also increased; for example, several communities initiated the construction of latrines. Volunteers appreciated being agents of change within their own communities. Participation levels were high, with an average of 70 participants per dialogue. The approach was particularly well received by women. Efforts to maximise prevention and control of schistosomiasis need to take community perceptions into account. Community dialogues are an effective tool to improve KAP and to increase community ownership of health issues. The approach has potential applicability in other health areas requiring communal behaviour change in resource-poor settings.

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EXPERIENCES WITH A URINE-BASED RAPID DIAGNOSTIC TEST FÜR *SCHISTOSOMA MANSONI* INFECTION IN MIGRANTS

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Schistosomiasis affects more than 250 million people, mainly in sub-Saharan Africa. Intestinal schistosomiasis is primarily caused by *Schistosoma mansoni*. Morbidity in chronically infected individuals can be subtle, but severe long-term consequences may arise (e.g. hepatic fibrosis). While the diagnosis of intestinal schistosomiasis relies on stool microscopy, it is important to note that low-intensity infections are easily missed. Here, we present experiences with a new point-of-care (POC) test that detects a schistosome-specific circulating cathodic antigen (CCA) in the urine of infected individuals. Since March 2015, individuals presenting to the University Medical Center in Homburg, Germany were tested for schistosomiasis upon specific infectious disease consultation. If the patients had lived in an endemic area and signs and symptoms were compatible with a parasitic infection (e.g. abdominal pain, eosinophilia), a urine sample was subjected to a POC-CCA test and stool and urine microscopy for parasites were performed. Patients with confirmed schistosomiasis were treated with praziquantel. Within 12 months, eight patients with a positive POC-CCA urine test have been identified. Upon further investigation, *S. mansoni* eggs were detected by stool microscopy in six of these patients. Of note, examination of a single stool sample failed to detect the infection in four patients. All individuals with confirmed schistosomiasis were migrants from Eritrea (age range: 16-34 years). Peripheral blood eosinophilia was present in three patients and ranged between 6% and 34%. Follow-up samples were obtained from three patients and gave a negative result on the POC-CCA test within 7-10 days after treatment. Our findings suggest that a POC-CCA urine cassette test is highly sensitive for detection of intestinal schistosomiasis in migrants from endemic areas. The high rates of migration into Europe and elsewhere will likely lead to an increase of imported schistosomiasis cases. Hence, a wider implementation of POC-CCA tests in hospitals will contribute to an improved diagnosis and management of otherwise undetected *S. mansoni* infections.

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ASSOCIATION OF SCHISTOSOMIASIS WITH IMPAIRED FERTILITY IN EAST AFRICA

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Many case reports and pathology series have suggested associations of Female Genital Schistosomiasis of the Fallopian tubes with infertility and ectopic pregnancy. Geographic distribution of infertility (which in Africa is most commonly due to tubal disease) has been reported but not explained. In this cross-sectional study, interpolated prevalence maps for *Schistosoma haematobium* and *S. mansoni* in East Africa were created using data from two open-access Neglected Tropical Diseases databases. Prevalence was extracted to georeferenced survey sample points for Demographic and Health Surveys for Ethiopia, Kenya, Tanzania and Uganda for 2009-2011 and 1999-2001. Outcomes included primary and secondary infertility (no births) and infecundity (no pregnancies) and history of pregnancy loss. Exploratory spatial analyses of outcomes (Moran's I, univariate and bivariate Local Indices of Spatial Autocorrelation) showed that outcomes were not spatially random and mapped clustering, hotspots, and areas of co-location of outcomes and exposures. Weighted multilevel logistic regression analysis found that women living in high compared to absent *S. haematobium* locations had significantly higher odds of secondary infertility (1999-2001: OR 1.8 [CI95 1.4, 2.3]; 2009-2011: OR 1.23 [1.1, 1.5]) and of primary infertility (1999-2001: OR 1.8 [1.3, 2.7]; 2001-2011: OR 1.58 [1.1, 2.3]). Living in high compared to absent *S. mansoni* locations did not affect the odds of any outcome. Women living in high *S. haematobium* compared to high *S. mansoni* locations had significantly higher odds of secondary infertility (1999-2001: OR 1.7 [1.3, 2.3]; 2009-2011: OR 1.6 [1.1, 2.0]), and of primary infertility (2010: OR 2.7 [1.5, 4.9]). For 1999-2001, history of pregnancy loss was significantly associated with high compared to absent *S. haematobium* (OR 1.3 [1.1, 1.6]) and with high *S. haematobium* compared to high *S. mansoni* (OR 1.4 [1.0, 1.8]). There is increasing evidence of the clinical and public health consequences of schistosomiasis to women's health and the importance of inclusion of girls and women in control strategies.

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REDUCED EFFICACY OF PRAZIQUANTEL AGAINST *SCHISTOSOMA MANSONI* IS ASSOCIATED WITH MULTIPLE-ROUNDS OF MASS DRUG ADMINISTRATION: EPIDEMIOLOGICAL AND GENOMIC DATA FROM UGANDA

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Mass drug administration (MDA) with praziquantel is the cornerstone of schistosomiasis control in sub-Saharan Africa. The effectiveness of this strategy is dependent on the continued high efficacy of praziquantel, however drug efficacy is rarely monitored using appropriate statistical or genomic approaches that can detect early signs of wane. We conducted a repeated cross-sectional study, examining children infected with *Schistosoma mansoni* from 6 schools in Uganda that had previously received between 1 and 9 rounds of MDA with praziquantel. We collected up to 12 *S. mansoni* egg counts from 414 children aged 6-12 before and 25-27 days after treatment with praziquantel. We estimated individual patient egg reduction rates (ERRs) using a statistical model to explore the influence of covariates, including the number of prior MDA rounds. In addition we sequenced whole-genomes of *S. mansoni* parasites before and after treatment. The average ERR among children within schools that had received 8 or 9 previous rounds of MDA (95% Bayesian credible interval (BCI) 88.23%, 93.64%) was statistically significantly lower than the average in schools that had received 5 (95% BCI 96.13%, 99.08%) or 1 (95% BCI 95.51%, 98.96%) round of MDA. We estimate that 5.11%, 4.55% and 16.42% of children from schools that had received 1, 5, and 8/9 rounds of MDA respectively had ERRs below the 90% threshold of optimal praziquantel efficacy set by the World Health Organization. The genomic population structure of parasites collected before and after treatment were compared to elucidate targets of selection against praziquantel. The reduced efficacy of praziquantel in schools with a higher exposure to MDA may pose a threat to the effectiveness of schistosomiasis control programs. We call for the efficacy of anthelmintic drugs used in MDA to be closely monitored.

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THE PREMONITION TRAP: LABORATORY TRIALS OF A ROBOTIC SMART TRAP FOR MOSQUITOES WITH SPECIES AND SEX RECOGNITION

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Using traps for surveillance of vector and pathogen populations is an important component of many vector management programs, but this technology has remained largely unchanged over many years. It is inaccessible in developing countries and limited in developed countries, largely due to costs and personnel requirements. Project PREMONITION is developing a robotic "smart" mosquito trap that captures live mosquitoes individually for higher-throughput lower-cost pathogen surveillance and elucidation of host associations. The PREMONITION trap uses infrared sensors and algorithms to identify flying insects by wing beat frequency, capturing only target mosquito species and reducing non-targeted captures. In addition to recording putative species identification, additional parameters including precise time of capture, temperature, humidity and ambient light, are recorded, effectively providing foraging activity data throughout the collection period and association with key abiotic factors. Due to a unique design, each specimen is tagged with the data it produced, enabling new bioinformatic analyses. Greenhouse trials of the PREMONITION trap with *Aedes aegypti* and *Culex quinquefasciatus* and different bait regimes including CO_2, skin odor and UV light evaluated the ability to trap mosquitoes, test behavioral activity throughout the collection period and differentiate between these taxa. Field testing will further evaluate the PREMONITION trap under native environmental conditions and exposure to a myriad of flying arthropods.

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REFINING ESTIMATES OF DENGUE VIRUS TRANSMISSION POTENTIAL IN WILD TYPE AND WMEL-INFECTED *AEDES AEGYPTI*: FIELD REARING CONDITIONS ALTER VIRUS SUSCEPTIBILITY

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Wolbachia- (wMel) infected *Aedes aegypti* have been released at field sites in Australia, Southeast Asia and now South America, with the intention of curbing dengue virus (DENV) transmission using wMel's virus inhibition phenotype. Under optimal rearing conditions in the laboratory, wMel induces reliable protection against DENV. However, mosquitoes reared in the field are subject to changeable conditions (eg: exposure to pathogens, fluctuating temperatures, variable nutrition/rearing densities), and such factors may alter wMel-induced blocking of DENV infection. We aimed to quantify the effect of field and laboratory rearing conditions on DENV susceptibility of wild type (WT) and wMel-infected *Ae. aegypti*, and to examine how this might influence virus transmission dynamics. Our approach utilised weekly deliveries of both WT and wMel mosquitoes collected directly from our Vietnamese field site. 'Lab-reared' eggs were hatched and reared under standard laboratory conditions. 'Field-reared' 4th instar larvae and pupae completed only the final stages of development (without added nutrition) in the laboratory. All adults were fed in parallel with the blood of 33 NS1-positive dengue patients admitted to the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam. As expected wMel mosquitoes were less susceptible to DENV than WT females. At the level of abdomen infection, logistic regression subgroup analysis showed that field rearing increased virus susceptibility in WT females, but did not affect wMel females. For virus transmission, WT field females more frequently transmitted virus in their saliva compared to lab-reared counterparts, suggesting lab experiments underestimate WT transmission potential in the field. The opposite held true for wMel females; wMel field females were less susceptible to DENV transmission than those reared in the lab, suggesting previous lab estimates of wMel-induced virus protection are conservative.

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ESTIMATING HUMAN/MOSQUITO CONTACT AND RISK OF EXOTIC ARBOVIRUS TRANSMISSION FROM EGGS COUNTS IN OVITRAP: A CASE STUDY FOR *AEDES ALBOPICTUS* IN ROME (ITALY)

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Aedes albopictus public health relevance is not only associated to its aggressive daytime biting behaviour, but also to its capacity to transmit arboviruses, such as Chikungunya (CHIKV), Dengue and Zika. The Zika epidemics occurred in 2015-16 in Latin America, in addition to the large number of Dengue cases/year worldwide, increases the possibility of virus importation to non-endemic countries where the species has now become a permanent pest. The likelihood of vector-borne disease transmission and spread is commonly estimated by assessing R0, i.e. the number of secondary infection arising from a primary case. However, R0-models heavily rely on the accuracy of estimates of relevant biological parameters for *Ae. albopictus* which may be very difficult to be determined and universally applied. We here exploited the most easily obtainable field data on *Ae. albopictus* density and dynamics (i.e. egg counts in ovitraps) to estimate one of the key epidemiological parameters for risk model (i.e. human/mosquito contact). Based on results from extensive field activities carried out in summer 2014 in two sites in Rome - one of the most heavily *Ae. albopictus* infested urban areas in Europe - we found a positive relationship between counts of eggs in ovitraps (N=25120) and host-seeking females collected by Human Landing Catches (HLC; N=5578). The estimated linear regression coefficient for the mean number of eggs/site/day was 0.21, meaning that an increase of 10 in the mean number of eggs in ovitraps corresponded to an increase of 2 in the mean number of daily host-seeking *Ae. albopictus*. Computation of R0 for CHIKV using observed HLC data showed values >1 in several periods along the sampling season. The computation of CHIKV outbreak probability highlighted two phases with a >75% probability of successful transmission of imported CHIKV. The approach proposed, if validated in different ecological/geographical settings, would represent a valuable and affordable option for assessing risk of exotic arbovirus transmission in non-endemic countries based on ovitrap data gathered during routine *Ae. albopictus* monitoring activities.

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IMPACT OF SEASONAL PATTERNS AND PARASITE ASEXUAL STAGE ON *ANOPHELES GAMBIAE* SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM* INFECTION IN BURKINA FASO

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Transmission reduction is a key component of global efforts to control and eliminate malaria. A wide range of novel transmission-reducing drugs and vaccines are currently under development. Human to mosquito transmission is influenced by many factors. Actually, it is unclear how the densities of parasites stages or season influence infection rate and intensity. Here, we describe the importance of the parasites stages seasonal pattern in infections success. Gametocytes carriers' infectiousness to mosquitoes was determined at the peak/end of transmission season and dry season via membrane feeding assay. Asexual parasites prevalence was higher in the peak of the wet season (69.1%, 329/476) compared to the dry (56.8%, 50/88) and the end of the wet season (60.5%, 161/266). Of gametocytes positive slides (N=189), 86.2% had asexual parasites. Gametocytes prevalence did not significantly vary between seasons. Asexual forms median density was 993 (IQR: 317-3759) with a significant difference between seasons (p=0.0004). However gametocytes median density 40 (IQR: 16-78) did not shown any seasonal variation (p = 0.1). For feeding, around 28062 mosquitoes offered blood meal and 29.6% fed and survived until dissection. The average number of dissected mosquitoes 75 (range 18 - 207) was quite the same according to the assays period. In 71.8% (79/110) of feeding experiments, at least one mosquito was infected. The median percentage of infected mosquitoes per infectious experiment was 15.7% (IQR: 07.3- 89. 2 %) with a median oocyst number of 2 (range 1 - 101). The prevalence of infected blood meal was similar across season (70.0%, 72.7% to 70.1% at the dry, the peak, and the end of the wet season. Mosquitos' infection rate also did not show any significant variation within season. The infection success was higher for asexual parasites carriers (91%) than non carriers (9%). However, mosquitoes' infection rate and oocyst load did not significantly vary according the asexual forms carriage. This highlights the need to carefully interpret evaluations, regarding asexual parasites and transmission season for malaria control program.

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INVESTIGATING THE INFLUENCE OF *PLASMODIUM* INFECTION ON THE HUMAN VOLATILE ODOUR PROFILE IN AN ENDEMIC SETTING

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There is some evidence to suggest that *Plasmodium* parasites manipulate the attractiveness of human hosts to *Anopheles* vectors. This is in accordance with the theory of parasite manipulation, whereby transmission is favoured by an effect of the parasite on host biology or behaviour. If malaria parasites can alter human attractiveness, the repercussions would be far reaching: this would likely have a profound influence on the way that malaria spreads through populations. Despite evidence that variations in human host attractiveness to biting insects are manifested through differences in the human odour profile, the association between such odour profile and malaria infection has never been studied. In this large-scale, field-based trial we used air entrainment techniques to capture the chemicals released by individuals' skin (the 'volatile odour profile', VOP). We measured the VOP of both uninfected school-age children in Western Kenya, and those with parasitaemia of both sexual and asexual *Plasmodium* stages. The same volunteers were measured following treatment with antimalarials. In addition to field diagnostics, parasite infection profile was characterised by the use of molecular techniques, including qt-NASBA and qPCR, for parasite stage and density respectively. Chemical odour profiles were examined using gas chromatography, then further investigated using coupled GC-electroantennography to determine entomological responses to constituent compounds. We will present results showing associations between human odour profile, parasitological parameters of infection and electrophysiological responses of the *Anopheles* vector. Malaria remains one of the most important diseases worldwide. As the global strategy for malaria control and elimination evolves to combat both parasite and vector resistance to drugs and insecticides, the need for innovative tools intensifies. Greater understanding of the factors that influence transmission will allow more precise epidemiological modelling. Additionally, measurable changes in the VOP of infected individuals could form the basis of a novel diagnostic tool.

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CHANGE IN MOSQUITO BEHAVIOR AFTER DISTRIBUTION OF BEDNETS RESULTS IN DECREASED PERSONAL PROTECTION FROM INFECTIVE BITES

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Behavioral resilience in mosquitoes poses a significant challenge to the control and elimination of malaria. Generalist host-seeking behaviors enable mosquito populations to evade indoor interventions which target host-seeking or resting mosquitoes. It is unclear whether behavior changes, as seen in anopheline vectors over the last decade, compromise the efficacy of vector control in reducing malaria transmission. In this study, we quantified human exposure to both bites and infective bites of a major malaria vector in Papua New Guinea over the course of four years surrounding a nationwide long-lasting insecticidal bednet distribution. Using age-stratified sleeping patterns and the biting density indoors and outdoors, we estimated the protective efficacy of LLINs against the biting population just prior to the distribution and for the next three years. We observed a shift in bite exposure to earlier hours of the evening, before individuals are protected, following the bednet distribution. Protective efficacy was greatest in children under five years old (65%), but significantly lower in the adult population (35%) who may be an important reservoir for transmission. The personal protection in all age groups decreased significantly over the study period and infective mosquitoes were found host-seeking before 10pm. As a result, exposure to infective bites was higher in net users and non-users alike, following the distribution. The result of the decrease in personal protection over several years appears to be a rebound in the entomological inoculation rate to levels that surpass pre-intervention estimates. This study highlights the necessity of validating and deploying vector control measures targeting outdoor exposure if malaria is to be controlled and eliminated.

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ACCELERATED ARBOVIRAL TRANSMISSION AND MORE EXPLOSIVE OUTBREAKS IN A WARMER WORLD

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Arboviruses are expanding into new regions, and outbreaks of these diseases are becoming more common. Several studies have quantified basic reproduction number, R0, based on temperature. However, none have quantified epidemic growth rate, r, an important temporal measure in the dynamics of these highly epidemic arboviral diseases. We seek to characterize the sensitivity of the epidemic growth rate to changes in both R0 and the generation interval, which is defined as the average time elapsed between consecutive cases in humans and also depends on temperature. We first developed a description of R0 for dengue based on temperature and, for the first time, a fully characterized generation interval distribution for dengue, also as a function of temperature. We then combined these estimates of R0 and the generation interval as functions of temperature to obtain a description of epidemic growth rate r as a function of temperature. To assess the implications of this temperature relationship for projected future temperature increases, we calculated r as a function of monthly, location-specific temperatures globally to identify areas of the world and associated populations that may be subject to increases or decreases in arboviral epidemic growth rates under future climate change. By 2050, as many as 3.2 billion people are projected to live in areas expected to experience increasingly rapid epidemics of arboviral diseases, indicating the need for surveillance systems to remain vigilant as the future explosiveness of outbreaks worsens in many areas.

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EXPOSURE OF EPITOPE RESIDUES ON THE OUTER FACE OF THE CHIKUNGUNYA VIRUS ENVELOPE TRIMER DETERMINES ANTIBODY NEUTRALIZING EFFICACY

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Chikungunya virus (CHIKV) is a reemerging alphavirus that causes a debilitating arthritic disease and infects millions of people and for which no specific treatment is available. Like many alphaviruses, the structural targets on CHIKV that elicit a protective humoral immune response in humans are poorly defined. Here we used phage display against virus-like particles (VLPs) to isolate seven human monoclonal antibodies (MAbs) against the CHIKV envelope glycoproteins E2 and E1. One MAb, IM-CKV063, was highly neutralizing (50% inhibitory concentration, 7.4 ng/ml), demonstrated high-affinity binding (320 pM), and was capable of therapeutic and prophylactic protection in multiple animal models up to 24 h post-exposure. Epitope mapping using a comprehensive shotgun mutagenesis library of 910 E2/E1 mutants with alanine mutations demonstrated that IM-CKV063 binds to an intersubunit conformational epitope on domain A, a functionally important region of E2. IM-CKV063 blocks both virus entry and release steps. MAbs against the highly conserved fusion loop have not previously been reported but were also isolated in our studies. The fusion loop MAbs were broadly cross-reactive against diverse alphaviruses but were non-neutralizing. Fusion loop MAb reactivity was affected by temperature and reactivity conditions, suggesting that the fusion loop is hidden in infectious virions. Visualization of the binding sites of 15 different MAbs on the structure of E2/E1 revealed that all epitopes are located at the membrane-distal region of the E2/E1 spike. Interestingly, epitopes on the exposed topmost and outer surfaces of the E2/E1 trimer structure were neutralizing, whereas epitopes facing the interior of the trimer were not, providing a rationale for vaccine design and therapeutic MAb development using the intact CHIKV E2/E1 trimer.

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BRIDGING THE GAP BETWEEN IMMUNOGENICITY AND SAFETY: AN INSECT-ONLY VIRUS AS A VACCINE PLATFORM

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Traditional approaches in vaccine development have typically involved live-attenuation or inactivation strategies. However, these approaches tend to offer unbalanced safety and immunogenicity profiles with live-attenuated vaccines providing robust yet reactogenic immune responses, while inactivated vaccines present a safer alternative often requiring multiple doses for protective immunogenicity. We propose the use of an insect-specific virus, Eilat virus (EILV), as a novel platform for alphavirus vaccines that provides exceptionally safe yet long-term, robust immunogenicity. EILV was isolated from a pool of mosquitoes collected in the Negev desert of Israel and is completely restricted to replication in insects only due to an inability to enter and replicate its RNA genome in vertebrate cells. To address the growing concern over chikungunya disease as it has now become a global threat, we developed a proof-of-concept chikungunya vaccine using the EILV platform. Here we report that EILV chimeras with chikungunya structural proteins are antigenically identical to their pathogenic counterpart, mimic early stages of the virus replication-cycle from attachment and entry to viral genome delivery, yet remain restricted to replication in mosquito cells only, providing an extremely safe phenotype in vertebrate animals with balanced, long-lived humoral and cellular immune responses following a single-dose vaccination. In non-human primates, EILV/CHIKV elicited rapid and robust neutralizing antibodies against chikungunya virus and provided protection against telemetrically-monitored disease. The EILV platform represents the first application of an insect-only virus in vaccine development and highlights the broader application of such viruses in vaccinology.

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PROTECTION AGAINST TWO LETHAL HETEROLOGOUS VIRUSES AFTER SIMULTANEOUS DUAL-AEROSOL CHALLENGE USING A NOVEL IMMUNOTHERAPEUTIC

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No specific therapeutic prevents or treats Venezuelan Equine Encephalitis (VEE) or other alphaviruses. VEE causes human epidemics and is a select agent. Influenza can cause severe mono-infection or a nosocomial co-infection in hospitalized patients admitted for other infections. Passive immunotherapeutics with mono-specific immunoglobulin (IgG) effectively treats toxin, venom and pathogen-mediated diseases and are either polyclonal IgGs, derived from human or animal plasma, or monoclonal antibodies. Effective multi-pathogen/toxin hIgGs could have great clinical benefits. Transchromosomic bovines (Tc-bovine) provide an alternative method to economically produce large quantities of multi-target IgG with fully-human antibodies. Tc-bovines have had their repertoire of bovine antibody genes deleted, and instead carry a human artificial chromosome containing the full repertoire of human antibody genes. Tc-

bovines can rapidly produce up to 600 grams per month of hyperimmune, multi-pathogen, fully-human immunoglobulin (hIgG). Tc-bovines were hyperimmunized with psoralen inactivated Trinidad-Tobago (Td-Tb) VEE virus and a trivalent spilt virion seasonal influenza virus (pH1N1, H3N2, type B) and a Tc-bovine hIgG with very high titers to these viruses was produced. Multiple groups of BALB-C or DBA2 mice (n=5) were aerosol challenged with 300 PFU of wild-type pDNA electroporated strain Td-Tb VEE (.5 PFU LD50) or dual challenged with 30 PFU Td-Tb VEE and 600 PFU pH1N1 (30 PFU LD50) on day 0. VEE aerosol challenged mice received 100 ug (5 mg/kg) IP at -12/+48h (prophylactic) or +12/48h (therapeutic). Dual VEE and pH1N1 aerosol challenged mice received 200 ug (10 mg/kg) IP at -12/+48h (prophylactic) or +12/48h (therapeutic). Controls received irrelevant hIgG. Prophylactic and therapeutic groups had 80-100% survival. All controls had 0% survival. To our knowledge, this is the first study demonstrating protection after simultaneous challenge with two lethal heterologous viruses (alphavirus + orthomyxovirus) by any route in any animal model. Multi-pathogen Tc-bovine hIgGs could be produced and tested in human clinical trials.

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A VIRUS-LIKE PARTICLE VACCINE ELICITS BROAD NEUTRALIZING ANTIBODY RESPONSES IN HUMANS AGAINST DISTINCT CHIKUNGUNYA VIRUS GENOTYPES

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Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that is endemic to many regions of Asia and Africa, and has recently emerged in the Western hemisphere. The rapid emergence of CHIKV is partly attributed to an adaptive mutation that facilitated efficient transmission by a widely distributed mosquito species, *Aedes albopictus*, highlighting the threat of further CHIKV spread and the need to prioritize vaccine development. There are three CHIKV genotypes: Asian, East/Central/South African (ECSA), and West African, which share 95.2 to 99.8% amino acid identity. CHIKV particles are enveloped, and encapsidate a positive-sense, single-stranded genomic RNA that encodes four non-structural and five structural polyproteins. Expression of the structural polyproteins gives rise to virus-like particles (VLPs), which are highly immunogenic. In a recent phase 1 trial, we demonstrated a CHIKV VLP vaccine based on a West African strain to be safe, tolerable, and to elicit robust neutralizing antibody (NAb) responses against an ECSA strain. Here, we investigated the breadth of the VLP vaccine-elicited NAb response against eight additional CHIKV strains representing all three genotypes to further evaluate the potential of this vaccine candidate to reduce CHIKV spread. We generated fully infectious chimeric viruses in which the non-structural genes of Semliki Forest virus (SFV), a related alphavirus, were complemented with CHIKV structural genes for use in neutralization assays against sera obtained from 12 CHIKV VLP vaccine recipients. SFV-CHIKV viruses encoding the structural genes from all nine CHIKV strains were infectious, demonstrated similar *in vitro* growth kinetics, and were potently neutralized by sera from vaccinees (average half-maximum inhibitory dilution per vaccinee: 2209, range: 315 - 6423). These results suggest that the CHIKV VLP vaccine-elicited NAb response could confer cross-protection against diverse CHIKV genotypes, further supporting the potential of this vaccine candidate to combat CHIKV spread. This vaccine is currently being evaluated in a phase 2 clinical trial in the Caribbean.

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HIGH-RESOLUTION IMAGING OF CENTRAL NERVOUS SYSTEM INVASION BY ALPHAVIRUSES DELIVERED BY SUBCUTANEOUS OR AEROSOL ROUTES OF EXPOSURE

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Encephalitic alphaviruses, including Venezuelan and Eastern equine encephalitis viruses (VEEV, EEEV), have caused sporadic outbreaks in the human population and can result in severe neurologic impairment and death. Although in nature virus is delivered subcutaneously by the bite of a mosquito, these viruses have potential for use as bioweapons, and in this case, they are likely to be delivered by aerosolization. Currently, no specific anti-viral treatments or FDA approved vaccines are available for VEEV or EEEV. Thus, a detailed understanding of the routes of brain entry and the dynamics of spread that differentiate the two will be important for the development of prophylactics or post-exposure interventions that may mitigate disease. In mouse models of disease, VEEV and EEEV demonstrate distinctly different cellular tropisms due to microRNA binding sites in EEEV that limit its growth in myeloid cells and natural heparan sulfate (HS) binding by the virus, which further limits myeloid cell infection but exacerbates brain replication. As a result of these phenotypic differences, the dynamics of neuroinvasion and spread within the brain are also expected to differ between the viruses. Using high speed ribbon scanning confocal microscopy and the prototypic strains VEEV Trinidad Donkey and EEEV FL93-939, we are collecting large-area, high resolution, images of brain tissue in three-dimensions. With these data we can resolve, at the cellular and macro levels, early sites of virus infection. Importantly, we can differentiate patterns of infection following different routes of exposure for both VEEV and EEEV. Initial results indicate that, while the timing of neuroinvasion differs between the two viruses, primarily dependent upon the HS binding phenotype, the olfactory bulb is a principal site of early infection for both viruses when delivered peripherally and via aerosol. We expect these data to provide a guide for the development of therapeutics that can be specifically targeted for the post exposure timing and route of infection.

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ATTACK RATE OF CHIKUNGUNYA IN NICARAGUAN CHILDREN DURING THE FIRST TWO WAVES OF THE EPIDEMIC, 2014-2016

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Chikungunya was recently introduced into the Americas, causing explosive epidemics throughout the region. Autochthonous transmission of Chikungunya was detected in September 2014 in Nicaragua. To study the introduction and transmission of Chikungunya in Nicaragua, we included testing for chikungunya virus (CHIKV) in an ongoing pediatric dengue cohort study in District II of Managua, the capital city. Children were enrolled prospectively, and data was systematically recorded on all medical visits. Participants are encouraged to come in at first sign of illness, and all medical care is provided free-of-charge through the study. Participants who present to the health center with suspected chikungunya or undifferentiated fever are tested for chikungunya by RT-PCR and serological assays. Between September 2014 and March 2016,

3,788 children participated in the study. From September 2014 to March 2015, the first wave of the epidemic, the clinical attack rate of laboratory-confirmed CHIKV infection was 2.9% (95% CI: 2.3%, 3.4%). From July 2015 to January 2016, the second wave of the epidemic, the clinical attack rate of laboratory-confirmed CHIKV infection was 13.9% (95% CI: 12.7%, 15.1%). Age was significantly associated with symptomatic CHIKV infection, with 2-4 year olds experiencing the lowest attack rate (9.4%) and 11-14 year olds experiencing the highest attack rate (20.2%). In the first year of the study, the proportion of children in the study with inapparent infections was 58.3% (95% CI: 51.5%, 65.1%). Poverty was an independent risk factor for CHIKV infection, with a prevalence ratio (PR) 1.33 (95% CI: 1.11, 1.58), as was having ≥8 hours per day without running water in the home, PR 1.31 (95% CI: 1.05, 1.63). The number of inapparent infections and the ratio of symptomatic:inapparent infections, along with risk factor analysis, is ongoing for the second wave of the epidemic. This study is providing critical data on the epidemiology and transmission of chikungunya in the Americas.

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TWELVE-MONTH ASSESSMENT OF PERSISTENT ARTHRALGIA ASSOCIATED WITH THE 2014-2015 CHIKUNGUNYA VIRUS OUTBREAK IN THE U.S. VIRGIN ISLANDS

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Chikungunya virus (CHIKV), an alphavirus transmitted by *Aedes* spp. mosquitoes, causes fever and polyarthralgia. Symptoms often resolve within 7-10 days; however, up to 79% of cases in previous outbreaks have reported persistent arthralgia (defined as joint pain at least once per week) for up to 36 months following acute infection. To enhance our understanding of the long-term impact of CHIKV illness, persistent arthralgia and functional outcomes of laboratory-positive cases in the U.S Virgin Islands (USVI) were evaluated at 6 and 12 months after symptom onset. Outcomes of individuals with similar healthcare seeking behaviors were compared to those of cases. A similar healthcare seeker was defined as a USVI resident who did not report experiencing sudden onset of fever and joint pain in June 2014-June 2015, and attended a healthcare facility during the last week of June 2015. Six months after illness onset, 165 cases (65% female, median age: 52) were interviewed and 12 months after illness onset, 128 of the 165 cases were interviewed. During the 12-month follow-up of cases, 167 similar healthcare seekers (64% female, median age: 34 years) were interviewed. The difference in prevalence of persistent arthralgia between cases and the comparison group at 6 months was 32% (95% confidence interval [CI]: 23-40%) after adjusting for age, sex and self-reported history of arthritis; at 12 months after onset, the difference in prevalence was 19% (95% CI: 11-28%). Twelve months after illness onset, cases were 1.81 (95% CI: 1.08-3.02) times more likely to have difficulty walking, 1.96 (95% CI: 1.24-3.12) times more likely to have difficulty climbing stairs and 2.63 (95% CI: 1.31-5.29) times more likely to have difficulty getting in and out of a car compared to similar healthcare seekers. Furthermore, 22% (95% CI: 15-29%) of cases compared to 10% (95% CI: 5-14%) of the comparison group reported that their health was either somewhat or much worse compared to one year prior. These findings highlight the long-term impaired physical functionality of CHIKV cases and the need for therapeutic and vaccine research to manage and prevent acute illness and long-term morbidity.

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HIGH SEROPREVALENCE OF MIDDLE EAST RESPIRATORY SYNDROME CORONAVIRUS (MERS-COV) IN CAMELS IS NOT ASSOCIATED WITH MERS-COV SERO-POSITIVITY AMONG CAMEL PASTORALISTS IN KENYA

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High seroprevalence of MERS CoV among camels has been reported in Kenya and other countries in Africa. To date, the only report of MERS CoV sero-positivity among humans in Kenya is of two livestock handlers with no known contact with camels. We assessed whether persons exposed to seropositive camels at household level had serological evidence of infection. A total of 798 human and 879 camel sera were collected from 265 households in Marsabit County in 2013. Household data collected included human and animal demographics, animal ownership and type of contact with camels. Human and camel sera were tested for antiMER-CoV IgG using a commercial ELISA test. Human samples were considered positive if positive on both ELISA and a confirmatory plaque reduction neutralization test (PRNT). PRNT was not performed on camel samples. Multivariable logistic regression was used to identify factors associated with MERS CoV sero-positivity and adjusted odds ratios (aORs) reported. The median age of persons sampled was 30 years (range 5-90) and 50% were males. A quarter (197/760) of the participants reported having had contact with camels defined as milking, feeding, watering, slaughtering or herding. Of the 798 human sera tested, 18 (2.2%) were positive on ELISA but negative by PRNT. Of the 879 camels sera tested, 90% (n=791) were positive. On multivariate analysis, older camels > 4 years and those raised under nomadic pastoral versus agro-pastoral production system had significantly increased odds (aOR 21.3; 95% CI 10.3, 43.6 and 18.5; 95% CI 7.4, 46.1, respectively) of being sero-positive, while the number of cattle in the herd and in households that had sold livestock were associated with decreased odds (aOR 0.9; 95% CI 0.96, 0.97 and 0.3; 95% CI 0.1, 0.9) of being seropositive. Despite high sero-prevalence among camels, there was no serological evidence of MERS CoV infection among camel pastoralists in Marsabit County. High seropositivity among camels suggests that MERS CoV or other closely related virus continues to circulate in camel herds particularly those raised in nomadic production systems, and highlights ongoing potential for animal to human transmission.

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REDRAWING THE BOUNDARIES OF KYASANUR FOREST DISEASE (KFD) IN INDIA- EARLY RESULTS OF GHSA-SUPPORTED ACUTE FEBRILE ILLNESS SURVEILLANCE

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Kyasanur Forest Diseases (KFD) is a tick-borne flavivirus disease first described in 1957 from the Shimoga district of Karnataka, India.KFD detection remained restricted to persons living in the Shimoga forest region until 2012 when it was identified in Chamarajanagar, 400km away.

Characteristically, this disease presents as an acute haemorrhagic febrile illness. In 2014 we initiated hospital-based laboratory-supported acute febrile illness (AFI) surveillance) at sub-district level sentinel hospitals across several states to map the aetiology of AFI in general, and KFD in particular. The project is supported under the Global Health Security Agenda (GHSA). All admitted AFI patients from June 2014 to March2016 with fever <15 days were enrolled. We recorded the demographic and clinical parameters of all cases, and tested for bacterial, viral and parasitic diseases, including leptospirosis, dengue, influenza, scrub typhus, chikungunya, typhoid, brucellosis, and KFD. Serological and molecular diagnostic assays were performed, including real-time PCR to detect viral RNA in serum for KFD confirmation. We enrolled 4693AFI patients from five contiguous Indian states. Of these, 302 (6.4%) were KFD-positive: Karnataka (98/2970), Kerala (45/1290), Tamil Nadu (1/29), Goa (143/391), and Maharashtra (15/15). KFD-positive patients ranged from 5 to 65 years (median age 40 years); 59% were female. Their clinical spectrum included myalgia (89%), generalized weakness (79%), prostration (22%), nausea (60%), vomiting (51%), abdominal pain (34%), diarrhea (24%), hemorrhagic fever (2%), and altered sensorium and or seizures (1%). Of 302 cases, 80% were living near the forest edge and 84% reported visiting the forest in the last 2 weeks. This study documents that KFD is not restricted to the Shimoga forest region, and has a more diverse clinical presentation than previously observed. Further, in our surveillance, we recorded cases without confirmed forest incursion.AFI platforms, as are being built under GHSA, are critical for the comprehensive characterization of known pathogens and may lead to detection of novel pathogens.

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RISK FACTORS FOR ACUTE LEPTOSPIROSIS IN NORTHERN TANZANIA

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Leptospirosis is increasingly recognized as a major cause of febrile illness in Africa but little is known about risk factors for human infection. Patterns of sero-reactivity in Tanzania have indicated that livestock may be important sources of human leptospirosis. To test this hypothesis we conducted a prospective cohort study of acute leptospirosis in northern Tanzania. We enrolled pediatric and adult patients with fever from two referral hospitals in Moshi, Tanzania and performed *Leptospira* microagglutination antibody testing on acute and convalescent serum. Cases were patients who had either a four-fold rise in *Leptospira* antibody titers or a single reciprocal titer ≥800, seropositivity required a single titer ≥100, and controls had titers <100 in both sera. We administered a standardized questionnaire to assess behaviors over the preceding month. We calculated odds ratios (OR) for individual behaviors, and combined behaviors to form exposure scales to livestock, rodents, and surface water. Of 1,446 patients enrolled from February 2012 through September 2014 the analyzed cohort included 24 (1.7%) cases, 179 (12.4%) seropositive participants and 592 (40.9%) controls. Among cohort members the median (range) age was 26.0 (0.2, 95.3) years and 422 (54.7%) were female. On bivariate analysis, acute leptospirosis was associated with age >12 years (OR 7.7, p<0.01), high level of cattle contact (OR 3.3, p=0.05), feeding cattle (OR 3.9, p=0.02), cleaning cattle waste (OR 4.3, p=0.03), working in fields (OR 2.9, p=0.02) and rice fields (OR 14.4, p<0.01). Seropositivity was associated with age >12 years (OR 2.2, p<0.01), Maasai ethnicity (OR 7.0, p=0.03), high level of cattle contact (OR 1.7, p=0.04), keeping cattle inside the house (OR 4.7, p=0.04), high level of goat contact (OR 1.9, p=0.03), slaughtering livestock (OR 1.7, p=0.02), and working in rice fields (OR 3.9, p=0.01). Behaviors and scales for exposure to rodents and surface water were not associated with either acute leptospirosis or seropositivity. Our findings suggest livestock contact and working in rice fields are important risk factors for leptospirosis in northern Tanzania.

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PRODUCTION FARMS, CLASS-1 INTEGRONS, AND ANTIBIOTIC RESISTANCE IN *E. COLI* ISOLATES FROM RURAL ECUADOREAN CHICKENS AND HUMANS

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While it is clear that industrial-scale farming operations affect antibiotic resistance (AbR) in livestock and humans, the extent to which smaller community-level farming operations impact AbR in surrounding communities, particularly in developing nations, is poorly described. We hypothesized that small-scale production operations contribute to elevated levels of AbR bacterial isolates in livestock and people living in close proximity to these operations by selecting for genetic mobile elements such as integrons that allow sharing of resistant gene cassettes between isolates. To investigate the relationship between community-level farming, mobile elements, and AbR, we conducted a serial cross-sectional community-based study collecting *E. coli* fecal isolates from over 1,000 chickens and humans in rural villages from northern Ecuador. Isolates were typed for 4 mobile elements using a DNA microarray platform; phenotypic resistance was assessed for 12 antibiotics using standard disc diffusion assays. We compared AbR levels in isolates from production chickens (broiler and laying hens raised for sale and fed commercial feed containing antibiotics) to AbR among isolates from household chickens (raised for domestic use and fed antibiotic-free feed). AbR for all markers was higher in production chickens compared to household chickens. There was a significant downward trend in AbR levels across birds from production operations, household birds in villages with production operations, and household birds in villages with no production operations, suggesting that proximity to a production operation can influence AbR in non-production chickens. In addition, the risk of phenotypic AbR appears to be modified by the presence or absence of class-1 integrons, suggesting a complex relationship between the environment, integrons, and AbR. These analyses (which will be expanded to include humans in houses associated with production or household chickens) suggest that small-scale production poultry farming selects for class-1 integrons in both chicken types, which has important public health implications.

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IDENTIFYING CHALLENGES AND OPPORTUNITIES FOR ONE HEALTH SYSTEMS STRENGTHENING IN GUINEA

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The recent Ebola virus disease outbreak in West Africa highlighted the importance of emerging zoonotic diseases, and provided an impetus for renewed emphasis on "One Health" capacity building with respect to zoonotic disease control. To guide capacity building efforts in Guinea, we sought to first identify the existing systems and structures in place for zoonotic disease control, specifically highlighting existing multi-sectoral approaches. Adapting our methodology for compatibility with the Global Health Security Agenda targets, we worked with the government ministries responsible for human, animal, and environmental health to identify a list of diseases - rabies, anthrax, brucellosis, viral hemorrhagic fevers (including Ebola fever, Lassa fever, yellow fever, and Rift Valley fever), trypanosomiasis and highly pathogenic avian influenza - as the Government of Guinea's top priorities. We then used each priority disease as a case study to identify existing processes for prevention, surveillance, diagnosis, laboratory confirmation, reporting and response across all sectors, with an emphasis on examining elements of cross-sectoral coordination or communication. Results were used to produce disease-specific systems "maps," which highlighted commonalities across all systems, as well as gaps and opportunities. Overall, we identified five major categories of gaps, each with a corresponding set of recommendations: 1) Coordination; 2) Training; 3) Infrastructure; 4) Public awareness; and 5) Research. These recommendations have been provided to the Government of Guinea to assist with the development of a One Health strategic plan. Overall, the project demonstrates an effective methodology for mapping systems and structures for zoonotic diseases, and the benefit of conducting a baseline review of systemic capabilities prior to embarking on capacity building efforts.

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PARASITES IN THE PARK: AN EPIDEMIOLOGIC STUDY OF NYC PARKS FOR *TOXOCARA* SPECIES

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Toxocara species are common pet parasites that can be found in the stool of dogs and cats. The infective larvae released in the stool survive in soil for many years and can subsequently be ingested by children who encounter them in sandboxes or on playgrounds. The CDC lists Toxocariasis as one of five neglected parasitic infections in the US and states that 'we urgently need to know more about who is at risk and how they are affected'. It is considered a neglected disease of poverty in the US, posing a significant disease burden in certain groups, remaining largely underdiagnosed. Infection in humans, paratenic hosts, can lead to visceral or ocular larva migrans, blindness, and silent brain infection that can diminish neurological cognition. New York City is pet-friendly, and known for its sprawling parks and play areas. It has been suggested that certain NYC neighborhoods may pose a higher risk for *Toxocara* infection, particularly in lower socioeconomic and predominately immigrant communities, although no study of actual *Toxocara* burden in NYC has been reported. The goals of this study are to:1)determine the burden of *Toxocara* in parks by examining sand/soil in playgrounds, 2) to determine if a disparity exists in neighborhood distribution, and 3) to explore which species of *Toxocara* is more prevalent. To accomplish these objectives multiple samples will be taken from more than 100 parks and playgrounds and analyzed by standard flotation and microscopy methods. Species will be identified by multi-parallel quantitative real-time PCR (qPCR) using specific primers to *T. cati* and *T. cani*. Parasite burden will be calculated. Results will be tabulated geospatially and correlated with US Census maps of income, housing and ethnicity. Preliminary results from 10 of 100 sites in this study identified *Toxocara* eggs in 40% of samples, suggesting that *Toxocara* is common in many play areas. At the conclusion of this project, we hope to provide the first epidemiologic geospatial survey of *Toxocara* species in NYC parks that may be helpful in identifying parks that may pose the highest risk of *Toxocara* transmission.

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USE OF TRACKING PLATES TO IDENTIFY HOTSPOTS OF RAT ABUNDANCE IN SLUM COMMUNITIES WITH HIGH ENDEMIC TRANSMISSION OF LEPTOSPIROSIS

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At present, there are no effective measures to prevent leptospirosis in slum environments, where the domestic rat is the primary reservoir. A critical barrier to mounting rodent control strategies is the lack of reliable approaches to estimate rat abundance and their distribution in complex urban settings. We developed a tracking plate method that quantifies the abundance of rats by identifying rat-specific markings on lampblack-coated tiles. We used this method to create high-resolution risk maps for rat abundance by placing tracking plates at 440 spatially randomized locations in a Brazilian slum community (0.46 km2) with high leptospirosis infection rates (35.4% CI, 30.7 - 40.6 per 1,000). We performed environmental surveys at sampling points and used satellite imagery to derive spatially relevant covariates. We analyzed the data using an interval-censored mixed model to evaluate the association between tracking board metrics and environmental characteristics. Among the 402 (91.4%) points that were successfully sampled, 173 (43%) had signs of rat-specific markings. Tracking plate-ascertained rat abundance was associated with the number of rat burrows (OR 1.32 CI 1.18 - 1.48), rat trails (OR 2.10 CI 1.8 - 2.62) and sites with rat feces (OR 1.39 CI 1.17 - 1.64). The distribution and intensity of rat abundance was highly heterogeneous. Clustering of rat abundance was identified throughout the study site and generally <20m in diameter. Clusters were associated with domestic areas (OR 1.47 CI 1.21 - 1.79) and areas with access to open sewers (OR 2.05 CI 1.69 - 2.48). Conversely, impervious surfaces (OR 0.55 CI 0.38 - 0.80) and increasing distance from flood-prone areas (OR 0.29 CI 0.17 - 0.50) and public trash dumps (OR 0.74 CI 0.60 - 0.96) were associated with decreased risk of rat abundance. By using tracking boards to create high-resolution risk maps, we identified defined environmental features of slum communities, which predict rat abundance. We also found that while rat abundance is high, there is marked spatial heterogeneity in the microenvironment, which may offer an opportunity for targeted rodent control for leptospirosis.

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FRACTION OF ALL UNDER FIVE DEATHS CAUSED DIRECTLY OR INDIRECTLY BY MALARIA IN SUB-SAHARAN AFRICA FROM 2000-2015

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Malaria cases have declined in sub-Saharan Africa due to the scale-up of interventions, and a consistent reduction in child mortality since 2000 has been recorded. In order to quantify the effect of malaria interventions on child mortality rates, we estimated the proportion of all-cause deaths attributed to malaria by direct and indirect causes. A detailed dataset of 978253 birth histories was assembled using cross sectional household surveys. The dataset included information on social and health factors. Birth history data were merged with *Plasmodium falciparum* prevalence rates and environmental and socioeconomic covariates obtained by remote sensing. Bias analysis was applied to identify confounders and effect modifiers among social and health covariates. A spatio-temporal hazard model was applied to estimate the relative risk of malaria for the surveyed communities. A structured additive regression (STAR) model was applied to generate a risk map for sub-Saharan Africa. The results of the STAR model were used to estimate the proportion of all-cause under 5 deaths due to malaria as direct and indirect cause at country level from 2000 to 2015. The proportion of all-cause deaths linked with malaria reduced drastically from 2000 to 2015. The reduction was more marked in Western and South Africa than in Central and Eastern Africa. Countries which have increased artemisinin-based combination treatments for children with non-complicated malaria showed a marked reduction of child deaths. The estimates of under 5 deaths indirectly or directly caused by malaria were higher than those calculated for malaria as direct one-cause-one death. The reduction of malaria transmission had significant impact on child mortality rates. The number of lives saved by the scale-up of malaria interventions was higher than previously estimated.

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THE DISTRIBUTION OF DHPS MUTATIONS IN AFRICA AND THEIR ASSOCIATION WITH DRUG PRESSURE AND TRANSMISSION INTENSITY

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Sulphadoxine-pyrimethamine (SP) was formerly widely used as a first-line treatment for *Plasmodium falciparum*, and continues to be important for prevention of malaria in pregnant women and children. Parasite mutations which confer resistance to SP spread earlier and more rapidly in some areas than others. Understanding why some areas are vulnerable to resistance could help inform surveillance and resistance prevention, and assess whether currently available data are sufficient to identify areas at risk. Here, we updated a systematic review on the K540E and A581G mutations in the dhps gene in Africa to 2016, previously completed in 2011. We geolocated each estimate, and linked each resistance data point with data from the same area and time, where available, on (a) slide prevalence, using Malaria Atlas Project estimates, and (b) SP use amongst febrile under five year olds, and coverage of intermittent preventive treatment in pregnancy (IPTp) from Demographic & Health Surveys and MICS Surveys, as well as SP market share from ACTWatch. Associations between resistance, drug pressure and transmission intensity were analysed using weighted regression. The systematic review resulted in 362 estimates of the prevalence of K540E and 220 A581G. In East Africa, the prevalence of both mutations generally increased or remained high, despite the end of first-line SP treatment policies. Here, the A581G emerged in locations where the prevalence of K540E was >50%. In West Africa, the K540E prevalence still did not reach levels as high as East Africa. In some sites where it emerged, later surveys no longer detected it. However, here the A581G mutation occurred when K540E was absent. We found a strong association between SP drug use in under-fives and K540E prevalence ($p<0.001$), but not A581G prevalence. Neither mutation was associated with IPTp coverage, nor SP market share data, nor transmission intensity. We are currently adding further covariates to the analysis, including age, presence of symptoms, and cotrimoxazole use. Our analysis could help inform surveillance for SP resistance mutations and potentially future emergent strains resistant to other antimalarials.

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PLASMODIUM FALCIPARUM AND *P. VIVAX* GAMETOCYTE CARRIAGE IN SOUTH AMERICA, ASIA AND THE SOUTH PACIFIC

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Reducing human-to-mosquito transmission is crucial to control and eliminate malaria, yet not everybody infected is infective to mosquitos. To identify those contributing most to transmission, cross-sectional surveys of the general population were conducted in Brazil, Thailand, Papua New Guinea and Solomon Islands, including 18,979 individuals. *Plasmodium falciparum* and *P. vivax* were diagnosed using microscopy and highly sensitive qPCR assays. Over 80% of individuals were asymptomatic, yet they accounted for 84% of *P. falciparum* and 86% of *P. vivax* gametocyte carriers. Blood-stage parasite density was the main predictor for gametocyte positivity in all surveys. Each 10-fold increase in parasite density resulted in a 1.5-fold and 3.9-fold increase in the odds of *P. falciparum* and *P. vivax* gametocyte positivity. For *P. vivax* a close correlation between parasite and gametocyte densities was found. By microscopy asexual stages and/or gametocytes were detected in 37-72% of individuals positive for *P. falciparum* gametocyte by RT-qPCR, and in 42-91% of *P. vivax* gametocyte carriers. Across all surveys, 95-99% of the total gametocytes biomass was found in microscopy positive samples, with no apparent correlation between transmission level and the proportion gametocyte carriers identified by microscopy. Microscopy is thus a valuable tool to identify asymptomatic infections contributing to malaria transmission. While in high transmission settings a large proportion of all gametocyte carriers and 85-99% of all gametocytes were found in children below 6 years, gametocytes were evenly distributed across all ages in low transmission settings. This suggests that interventions to reduce transmission in high transmission areas will have the greatest effect when targeted towards children, but in order to achieve elimination in low transmission settings individuals of all ages must be targeted.

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DIABETES AND OBESITY AS RISK FACTORS FOR SEVERE MALARIA: AN OBSERVATIONAL STUDY OF COMORBIDITIES IN *PLASMODIUM FALCIPARUM* CASES DIAGNOSED IN SWEDEN OVER 20 YEARS

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Non-communicable diseases and obesity are increasing global health problems, also in malaria endemic countries. The impact of these conditions on the severity of malaria needs to be established. The aim of this study was to assess if comorbidity, in terms of chronic diseases and obesity, is associated with severe malaria in adults with *Plasmodium falciparum*. Patients aged >18 years with *P. falciparum* malaria diagnosed at multiple sites throughout Sweden 1995-2015 were included in the study. Medical records were retrospectively reviewed regarding demographics, travel history, clinical presentation, weight, height, and comorbidity. Severe malaria was defined according to WHO criteria and hyperparasitaemia >5%. Obesity was defined as body mass index (BMI) ≥30, according to WHO classification. Data was analysed using multivariable logistic regression models. 937 patients with *P. falciparum* malaria were included in the study, 547 (58.4%) originated from endemic countries in Sub-Saharan Africa and 388 (41.4%) from non/low endemic countries. In total, 92 patients fulfilled the criteria of severe malaria, of which 22 (23.9%) had at least one comorbidity included in the Charlson Comorbidity Index compared to 84 (9.9%) among non-severe cases ($p < 0.001$). Age, health care delay, origin in non/low endemic country, diabetes, hypertension, cardiovascular disease, HIV and BMI ≥30 were associated with severe malaria in univariable analyses. In multivariable analysis adjusted for age, health care delay, patient origin and HIV, both diabetes and obesity were associated to severe malaria. Patients with obesity together with another metabolic risk factor (hypertension, dyslipidaemia or diabetes) had an even more pronounced risk of severe malaria in adjusted analysis. In conclusion, diabetes and obesity were independently associated with an increased risk of severe malaria in adults diagnosed with *P. falciparum* in Sweden. These metabolic comorbidities need to be considered in the acute management and prevention of malaria in adults. Moreover, their role in human malaria pathology needs to be further investigated.

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THE DESCRIPTIVE EPIDEMIOLOGY OF PEDIATRIC SEVERE MALARIAL ANEMIA IN MALI AND TANZANIA

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Severe malarial anemia, defined as hemoglobin <5 g/dL in the presence of a *Plasmodium falciparum* infection, is the most common manifestation of severe malaria among young children in regions with very high malaria transmission. However, basic questions persist about the natural history of this profound anemia and the factors that contribute to an individual's risk of developing this syndrome during malaria infections. Drawing on birth cohort data collected from over 75,000 study visits with 880 children in the Mother Offspring Malaria Studies Project (2002-2006) in Muheza, Tanzania, and 1647 children in the Malaria Research and Training Centre-Laboratory of Malaria Immunology and Vaccinology Immuno-Epidemiology Observational Study (2010-2016) in Ouélessébougou, Mali, we aimed to describe in detail the epidemiology and pathogenesis of severe malarial anemia in early life. While severe malarial anemia occurred at older ages in the seasonally endemic Malian study site (median (IQR): 63 (39, 100) weeks) than in the perennially endemic Tanzanian setting (median (IQR): 34 (29, 52) weeks), these data suggest that the pathogenesis of malarial anemia is largely conserved across transmission settings. We will present data describing the associations between severe malarial anemia risk and parasite density, baseline hemoglobin levels, and bioavailable iron in early life. We will also use individual case data to illustrate that, although a subset of cases arise from gradually declining hemoglobin profiles associated with repeated infections, severe malarial anemia more commonly presents as an acute drop in hemoglobin. From a public health perspective, these findings reinforce the value of campaigns designed to interrupt malaria transmission and to reduce high density infections.

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TRAVEL PATTERNS AND DEMOGRAPHIC CHARACTERISTICS OF MALARIA CASES: THE CASE STUDY OF SWAZILAND, 2010-2014

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As Swaziland gets closer to its 2018 national elimination goal, importation of parasites into receptive areas becomes increasingly important; imported infections have the potential to instigate local transmission and sustain local parasite reservoirs. Travel history (reported travel in the last 8 weeks) from Swaziland's routine surveillance data from 2010 to June 2014 was extracted to describe and compare travel patterns of RDT-confirmed index cases (identified passively) to travel patterns of individuals that tested negative (during re-active case detection, where the contacts in the vicinity of an index case within receptive areas were tested). Reported destinations of travel were geo-located to the smallest administrative boundary possible. Of 1,517 confirmed index cases 67% reported travel history, whilst 105 (1%) of the 9,859 contacts screened during the reactive case detection were RDT positive. 876 index cases travelled internationally. The proportion of index cases reporting travel history increased by year with a 48% increase per annum for international travel and 27% increase for travel within Swaziland. 25% of screened contacts reported international travel, of which 22% tested positive for malaria. Mozambique was the most likely travel destination of positive individuals, with Maputo City, Inhambane and Gaza being the most likely destinations in Mozambique. 97% percent of RDT positive international travellers were either Swazi (52%) or Mozambican (45%), however Swazis were more likely to test negative. All international travellers were unlikely to have a bed net at home or use protection while travelling. 84% of the 755 males and 60% of the 440 females that travelled abroad tested positive. Overall, 24 to 45 year olds were most likely to report travel. Additionally, paths of transmission, important border crossings and means of transport were identified. Results from this analysis can be used to direct national and well as cross-border targeting of interventions, over space, time and by sub-population. Collaboration between neighbouring countries is needed to tackle the importation of malaria at the regional level.

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PLASMODIUM KNOWLESI MALARIA IN CHILDREN IN MALAYSIA: NO SEVERE DISEASE DESPITE AN INCREASED RISK OF ANAEMIA COMPARED TO ADULTS

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Plasmodium knowlesi is now the most common cause of malaria in Malaysia, but prospective studies describing the clinical spectrum have only detailed adult disease. In our prospective study (2012-ongoing) at 3 district hospitals in Sabah, Malaysia, previously untreated, non-pregnant patients of any age hospitalised with PCR-confirmed malaria includes: 500 *P. knowlesi* (49 [9.8%] children ≤12 yrs), 204 *P. vivax* (83 [40.7%] children), 103 *P. falciparum* (33 [32%] children), 26 *P. malariae*, 1 mixed *Pk/Pf* and 1 mixed *Pv/Pf*. Preliminary data include a lower baseline parasite count for *P. knowlesi* malaria patients (median 2480/µL, IQR 538-8481) than *P. falciparum* (9600/µL [IQR 1446-25440]; $p<0.001$), and *P. vivax* (3882/µL, [1454-9608]; $p=0.016$). Parasite counts were higher in adults with *P. knowlesi* compared to children (median 2800 vs. 1535/µL; $p=0.019$). Anaemia (WHO criteria) was present in 32% (95%CI 27-36) of adults vs. 81% (95%CI 70-93) of children with knowlesi malaria ($p<0.001$); comparable to that seen in children with *P. vivax* (82%; $p=0.948$) or *P. falciparum* (76%; $p=0.551$). Acute kidney injury (AKIN criteria and/or creatinine >132mmol/L) was found in 26% (95% CI 22-30) of those with *P. knowlesi* malaria including 111/437 (25%) of adults and 13/46 (26%) of children ($p=0.673$). There was a higher prevalence of acute kidney injury in children with *P. knowlesi* malaria (26%) compared to *P. vivax* (12.5%; $p=0.027$) but not *P. falciparum* (30.3%; $p=0.844$). Severe malaria (modified WHO 2010 criteria) was present in 6.4% (95%CI 2.4-9.6%) of those with *P. knowlesi* overall: 32/451 (7.1%) in adults but none in children. In comparison, severe disease was seen in 6/204 (2.9%; $p=0.065$) with *P. vivax* and 4/103 (3.9%; $p=0.326$) with *P. falciparum*. There were no treatment failures in knowlesi malaria patients seen at 28 days. There was one *P. knowlesi* malaria death, an adult (age 62) with delayed parenteral artesunate due to misreported hyperparasitaemia. Overall *P. knowlesi* malaria predominantly affected adults, and while anaemia was more common in children, parasitemia was lower and severe disease was not seen.

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ASSESSMENTS OF WILDLIFE RESERVOIRS OF *TRYPANOSOMA CRUZI* AND THEIR INTERACTIONS WITH TRIATOMINE VECTORS ACROSS TEXAS

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Despite the importance of various wildlife species for perpetuating *Trypanosoma cruzi,* agent of Chagas disease*,* in nature, little is known about the relationship between infection and cardiac disease in wildlife, or the relative importance of wildlife species for feeding bugs. Using a cross-sectional study design, we collected cardiac tissue and blood from potential *T. cruzi* reservoirs across Texas, a state with widespread endemic, infected triatomines. Target species included migratory bats, nuisance rats (*Rattus rattus*), feral swine (*Sus scrofa*), and hunter-harvested predators including raccoon (*Procyon lotor*), coyote (*Canis latrans*), gray fox (*Urocyon cinereoargenteus*), and bobcat (*Lynx rufus*). Concurrently, we collected triatomines from regions of wildlife trapping and through a statewide citizen science campaign. Wildlife samples were tested for *T. cruzi* and parasite lineage was ascertained from the TcSD5D gene sequence. Vector-host interactions were determined using a bloodmeal analysis to amplify the vertebrate cytB gene in bug hindguts. No rats (n=152), a single bat (n=593), 5.6% of feral swine (n=54), 13.8-14.3% of bobcats, coyotes, and foxes (n=156), and 70% of raccoons (n=70) were infected with *T. cruzi*. The bat harbored lineage TcI, whereas TcIV was found in all raccoons that were typed. Although a histologic survey of right ventricles showed 21.1% of PCR-positive raccoons had mild lymphoplasmocytic infiltration, no other lesions were observed in raccoons, and none of the positive pigs showed cardiac pathology, suggesting infection is associated with minimal cardiac disease in these species. Diverse wildlife species were identified as bloodmeal hosts in an analysis of 76 bugs, including rats, raccoons, rabbits, feral swine, squirrel, fox, skunk, opossum, deer, and toads, but the majority of hosts were dogs (50%) and humans (22.4%), likely reflecting the nature of bugs encountered in the citizen collection program. Characterization of the robust sylvatic transmission cycles of *T. cruzi* combined with analyses of local triatomine feeding patterns will lead to ecological interventions to reduce Chagas disease risk.

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LEISHMANIA INFANTUM PARASITEMIA IN ASYMPTOMATIC BLOOD DONORS IN AN ENDEMIC REGION OF NORTHEAST BRAZIL

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Leishmania infantum causes fatal visceral leishmaniasis (VL), but a majority of infections are asymptomatic. Transmission by blood transfusion has been documented in Europe, but the potential impact of *L. infantum* in blood donations in Latin America has not been assessed. We hypothesized that blood donations contaminated with *L. infantum* would be found at the blood bank serving Natal, northeastern Brazil, a city with a high prevalence of periurban *L. infantum* infection. Blood donations were examined for occult *Leishmania* infection in the Natal blood center. Three-hundred blood units initially rejected on the basis of positive screening for pathogens including *T. cruzi* or insufficient volume, and 254 samples from routine blood donors were assessed for *Leishmania* by culture, qPCR, and serology (ELISA). Remarkably, 18 of 300 (4%) rejected blood units were culture positive for *L. infantum*. qPCR was positive for *Leishmania* in 8.7%, and 22% were seropositive for Leishmania. Anti-*Leishmania* antibody levels correlated with *Leishmania* load by qPCR. Of the 254 blood samples that tested negative for other pathogens, 28.4% were seropositive for *Leishmania*, 7.7% were qPCR positive and one was culture positive. *T. cruzi* ELISA detected only 14/18 culture positive, 38/107 seropositive, and 22/37 qPCR positive donations. In conclusion, asymptomatic *Leishmania infantum* infections are associated with infected blood donations in northeastern Brazil. DNA testing by qPCR seemed the most sensitive and specific donor screening method. Serological assays for *T. cruzi* detected many but not all of the Leishmania-infected donors, likely due to cross-reacting antibodies and/or dual infections. The data suggest a need to screen blood donations for *L. infantum* in residents of, and immigrants from endemic regions.

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CUTANEOUS AND MUCOCUTANEOUS LEISHMANIASIS IN INTERNATIONAL TRAVELERS: RESULTS FROM THE GEOSENTINEL SURVEILLANCE NETWORK

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Due to increasingly exotic travel, displacement of migrant populations, and expanding vector ranges, cutaneous leishmaniasis (CL) is emerging among international travelers and migrants, and limited data exist on mucocutaneous leishmaniasis (MCL) in travelers. We describe the epidemiology of travel-associated CL and MCL among international travelers and immigrants over an 8-year period through descriptive analysis of GeoSentinel data. Demographic and travel-related data on returned international travelers diagnosed with CL or MCL at a GeoSentinel Surveillance Network site between March 15, 2007 and August 31, 2015, were analyzed. Eight-hundred, twenty-eight returned travelers or new immigrants were diagnosed with CL or MCL during the study period, 824 (99.5%) of which were travel-acquired. Of travel-acquired cases, immigrants accounted for 8% (n=66). For all non-immigration travelers, the most common source countries were Bolivia (n=150, 19.8%) and Costa Rica (n=89, 11.7%), while for new immigrants, they were Afghanistan (n=18, 27.3%) and Syria (n=15, 22.7%). Eighty-one travelers (9.8%) acquired their disease on trips of ≤2 weeks. Species identification was available for 218 cases (26.5%). *Leishmania Viannia braziliensis* was the most well represented strain (n=93, 45.6%), followed by L. major (n=31, 15.2%), and *L. V. panamensis* (n=27, 13.2%). Thirty-five cases of MCL occurred, most of which were in tourists (n=26, 74.3%) and acquired in Bolivia (n=16, 45.7%). CL is predominantly a disease of tourist travelers to areas such as Bolivia where risk of acquiring L. V. braziliensis and subsequent MCL is high. That many travelers acquired their illness on trips lasting ≤2 weeks challenges the common notion that CL is a disease of prolonged travel. New immigrants from areas of conflict and political instability, such as Afghanistan and Syria, were well represented, suggesting that as mass migration of refugees continues, CL will be increasingly encountered in intake countries. Initiatives to enhance awareness and assure adequate resources for diagnosis and management of leishmaniasis are needed.

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ASSOCIATIONS BETWEEN PARASITOLOGICAL AND SEROLOGICAL INDICATORS OF INFECTION AND THE DEVELOPMENT OF CLINICAL VISCERAL LEISHMANIASIS IN ETHIOPIA

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Visceral leishmaniasis (VL) is a vector-borne parasitic disease that results in 40,000 deaths annually. In East Africa, human infection by *Leishmania donovani* occurs via anthroponotic transmission, while the role of zoonotic routes remains unclear. Increased risk for VL is associated with malnutrition, male sex, and animal ownership. A recent upsurge in case counts and spatial spread of the disease in Ethiopia have demonstrated the need for more effectively implemented control policies. Data from a prospective cohort study conducted between 2011-2013 in northern Ethiopia provided longitudinal distributions of infection intensities (assessed by qPCR) and rates of seropositivity (assessed by direct agglutination test, DAT). Multivariate logistic regression with model selection allowed for evaluating independent risk factors for *L. donovani* exposure, seropositivity, and development of clinical VL. At baseline, 14.1% of 4,722 individuals had >0 parasites per ml of blood and 3.3% were seropositive at DAT titers >1:800. Despite similar distributions of infection intensity to females, males were significantly more likely to be seropositive during the study (6.8% vs 3.6%, P<0.001). After adjustment for age and body mass index, seropositivity was associated with infection intensities >100 parasites per ml blood. Seventy-five incident VL cases were recorded during the study period. Progression to clinical disease was significantly related to male sex (OR: 1.68, 95% CI: 1.01, 2.80), DAT positivity (OR: 4.60, 95% CI: 2.25, 9.41) and high infection intensity, specifically 101-1000 parasites per ml blood (OR: 4.67, 95% CI: 1.80, 12.13). Significantly increased odds of infection, seropositivity, and clinical disease were associated with having a seropositive household member. Simultaneous seropositivity and high infection intensity were significantly associated with progression to clinical VL. Males tended to exhibit these risk factors more than females. Identifying people with DAT titers >1:800 and then performing routine qPCR to determine the subset with high infection intensity could be a strategy for targeted intervention.

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PROGRESSION AND MORTALITY RATES FOR MODELLING THE BURDEN OF CHAGAS DISEASE

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Accurate estimates of morbidity and mortality due to Chagas disease are needed to improve burden of disease evaluations. For parasitic infections, disease burden models link infection frameworks with associated morbidity (sequelae) and mortality, a much needed area of research for a better understanding of the impact and cost-effectiveness of control interventions. Such modelling efforts require elucidating the relationship between infection and disease as well as its rigorous and robust parameterisation. A systematic review was conducted to identify observational (longitudinal) studies comparing disease progression and mortality rates in populations with and without Chagas disease. Literature databases were searched without restrictions on publication language or date; 8,935 potentially relevant references were screened. Information on

selected papers was extracted and analysed using a random-effects model for both disease progression and mortality rates. The results of a previously developed force-of-infection model (for Colombia), yielding incidence and prevalence trends were linked to the progression and mortality rates thus quantified. This disease model was used to calculate the Disability Adjusted Life Years (DALYs) attributed to Chagas disease in Colombia. For assessment of progression rates, 19 studies were selected which provided 49,792 patient-years of follow-up. The general progression rate was 2% per year (95%CI: 1.7-3.4). For mortality rates, 25 studies were selected, providing data on 53,346 patient-years of follow-up, and 2,739 events. Pooled estimates revealed that Chagas disease patients have significantly higher annual mortality rates (AMR) compared with non-Chagas disease patients (0.18 vs. 0.10; RR = 1.74, 95 % CI 1.49-2.03). The application of these progression and mortality rates to burden of disease models allows us to estimate figures and burden metrics by clinical stage. A more refined analysis would allow us to estimate progression to megaesophagous, stroke and other outcomes.

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DEFINITIONS AND FEASIBILITY OF ELIMINATION OF VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is the second deadliest parasitic disease globally and has been targeted by the WHO for elimination and control by 2020. Two separate modeling approaches have been used to look into the feasibility of reaching the targets with current intervention strategies. 'Elimination of VL as a Public Health problem', the target for the Indian subcontinent, is predicted feasible in low and medium endemic regions with optimal implementation of interventions. However, highly endemic areas and regions with suboptimal interventions are likely not to achieve the target on time and will require additional efforts. 'Elimination of Disease', the target for the rest of the world, can be achieved by more regular serology testing, to identify which individuals are likely to develop clinical symptoms, so that they can be treated promptly. This should reduce the intensity of transmission and could lead to 'Elimination of Transmission'. The remaining knowledge gaps in the disease dynamics of VL, such as the contribution of asymptomatic individuals to transmission, present a challenge to reaching and sustaining the elimination targets.

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VISCERAL LEISHMANIASIS IN THE INDIAN SUBCONTINENT: HOW MUCH DO ASYMPTOMATICS CONTRIBUTE TO TRANSMISSION AND HOW DOES TRANSMISSION DECREASE WITH DISTANCE FROM A CASE?

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A key unknown in the transmission of visceral leishmaniasis is how much asymptomatically infected individuals contribute to transmission. Since they significantly outnumber individuals who develop clinical symptoms (by 4-10 times in the Indian subcontinent) and are often infected for a long period, they may contribute substantially to transmission even if their relative infectivity to sandflies is low. This has important implications for attempts to control and eliminate the disease, as there is currently no safe treatment for asymptomatic infection, and interventions are focussed on reducing transmission through rapid diagnosis and treatment of symptomatic cases combined with indoor residual spraying of insecticide in endemic areas. Efforts to determine the infectivity of asymptomatic and symptomatic individuals via xenodiagnostic trials are ongoing, but these critical parameters are not known. Another unknown factor that has major implications for control is how the risk of transmission varies with distance from infectious individuals. This is key to determining whether responsive insecticide spraying strategies, in which houses within a certain radius of an index case are sprayed, will reduce transmission. To start to address these questions we have developed an individual-based spatiotemporal model of visceral leishmaniasis transmission and fitted it to detailed epidemiological and serological data from three highly endemic villages in Bangladesh to estimate the infectivity of asymptomatic individuals and the spatial kernel of transmission. Using Bayesian MCMC methods we have been able to account for the unknown times of infection of asymptomatic and symptomatic individuals, variation in infected individuals' infectivities over time, and potential false positives from serological tests. Our results suggest that the contribution of asymptomatic individuals to transmission in this highly endemic setting was small compared to that of individuals with clinical VL, and that the risk of infection was greatest for individuals within 40m of a VL case within 6 months of their onset of symptoms.

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DOES ARTIMISININ BASED COMBINATION THERAPY INFLUENCE MOSQUITO FITNESS AND HOST-SEEKING BEHAVIOR?

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Artemisinin-based combination therapy (ACT) is recommended against malaria in many endemic areas, and thus widely used. Surprisingly little is known about the effect of ACTs on mosquitoes that transmit malaria parasites. Our objectives were to (1) determine whether ACTs have a direct impact on mosquito fitness when added to the bloodmeal, and (2) evaluate whether ACTs influence mosquito host-seeking behavior by changing the intrinsic attractiveness of human skin odor. Our study was done with *Anopheles gambiae* s.l., which is the main vector of malaria in sub-Saharan Africa. Mosquitoes that were fed on blood with ACT survived equally long as mosquitoes fed on control blood. ACT-fed and control mosquitoes also laid equal numbers of eggs, thus fitness was not affected by the treatment. To investigate host-seeking behavior of mosquitoes, adult malaria-free men were given a treatment dose of an ACT, and skin odor was collected on nylon socks before, during and three weeks after treatment. A screenhouse choice test showed no preference of *An. gambiae* females between socks worn by the same person before, during or after ACT-treatment. Relative attractiveness of nylon socks to An. coluzzii in a dual-choice olfactometer was also not influenced by ACT-treatment although mosquitoes appeared to be more responsive to skin odor collected three weeks after ACT-treatment. We conclude that ACT-treatment does not affect fitness and host-seeking behavior of malaria mosquitoes. Our results are important in light of possible transmission of gametocytes from ACT-treated people to malaria vectors.

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UNEXPECTEDLY LOW HUMAN BLOOD INDEX ASSOCIATED TO HIGH *PLASMODIUM* SPOROZOITE RATES IN *ANOPHELES GAMBIAE* COMPLEX SPECIES FROM A LLIN-PROTECTED VILLAGE IN BURKINA FASO

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The global effectiveness of long-lasting insecticidal nets (LLINs) in reducing malaria transmission is indisputable. However, in areas where malaria transmission levels are extremely high, substantial reductions in transmission intensity only led to a modest reduction in human parasitaemia. A paradigmatic case is represented by Burkina Faso where, after a few years of mass distribution of LLINs, the burden of malaria has not significantly changed as highlighted by WHO Country statistics and statistical bureau of Burkina Faso. We here report the results of a longitudinal survey on host choice and *Plasmodium* sporozoite rate (SR) in malaria vectors belonging to *Anopheles gambiae* complex in a rural village of Burkina Faso where LLINs were broadly distributed the year before the sampling (August - November 2011). The human blood index (HBI) was 18.8% (N=112) and 8% (N=75), in An. coluzzii and An. arabiensis, the two most abundant malaria vectors in the area. These values are much lower than usually reported particularly for An. coluzzii, which is known as a highly anthropophilic species, but consistent with the hypothesis that LLINs reduced the availability of human hosts to mosquitoes. Unexpectedly, *Plasmodium* sporozoite rates (*An. coluzzii*: 7.6%, N=449; *An. arabiensis*: 5.2%, N=229) were found to be in the range of those reported in the region before LLIN implementation when much higher HBIs were observed. This suggests that, despite LLINs have significantly reduced human/vector contact, this has not apparently yielded to a substantial reduction of mosquito infection rates. Further investigations are needed to confirm these results; however, they are fully consistent with the lack of effectiveness of LLINs in stemming malaria transmission in the study area.

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THE WMEL STRAIN OF WOLBACHIA REDUCES TRANSMISSION OF ZIKA VIRUS IN *AEDES AEGYPTI*

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Zika virus (ZIKV) is an arbovirus that belongs to the family Flaviridae. It currently is causing an explosive outbreak of febrile disease in the Americas. During the current outbreak, a causal relationship has been established between prenatal ZIKV infection and microcephaly and other serious brain anomalies. Most human cases around the globe result from ZIKV emergence into a human-mosquito cycle involving *Aedes aegypti* and/or other urban or peri-urban *Aedes* species. Despite the continued spread of the virus, there remain no effective antiviral therapies or licensed vaccines. Thus, with its continued invasion of the new world, the only tools presently available to combat Zika target mosquito populations, mostly with insecticides and larval source reduction. But, these strategies have not prevented invasion of this virus into new locales and have not been adequate to control the virus upon arrival. A promising candidate for arbovirus control and prevention relies on the introduction of the intracellular bacterium Wolbachia into *Ae. aegypti* mosquitoes. This primarily has been proposed as a tool to control dengue virus transmission; however, evidence suggests Wolbachia infections confer protection for *Ae. aegypti* against chikungunya virus as well. Although this approach holds much promise for limiting virus transmission, at present our understanding of the ability of ZIKV to infect, disseminate, and be transmitted by wMel-infected *Ae. aegypti* currently being used at Wolbachia release sites is unknown. Using *Ae. aegypti* infected with the wMel strain of Wolbachia that are being released in Medellin, Colombia, we report that these mosquitoes have reduced vector competence for ZIKV. In fact, we were not able to detect infectious ZIKV in the saliva of mosquitoes at any timepoint assayed. These data argue for the expansion of this technology to ZIKV in South and Central America and are useful and germane in the broader context of ZIKV-mosquito interactions. Finally, we also describe a biologically relevant model for studying ZIKV transmission dynamics (feeding on a viremic host) that does not rely on animal blood spiked with cultured virus.

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COMBINING CONTACT TRACING WITH TARGETED INDOOR RESIDUAL SPRAYING SIGNIFICANTLY IMPACTS DENGUE TRANSMISSION

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The current paradigm for dengue virus (DENV) control relies on reactive measures aimed at containing virus transmission around the home residence of confirmed symptomatic cases. Unfortunately, the impact of such strategy on preventing virus transmission is very limited. Furthermore, the recent emergence of Chikungunya and Zika infections in the Americas elevates the need for more efficacious tools for virus surveillance, *Aedes aegypti* control and disease mitigation. We quantitatively investigated the epidemiological value of performing location-based contact tracing (incorporating potential out-of-home exposure locations by phone interviews associated with ongoing DENV passive surveillance) in improving identification of local dengue transmission foci across a metropolitan area (Cairns, Australia). Using space-time interaction tests applied to 2,064 potential exposure locations reported by contact tracing from 902 DENV-confirmed cases we statistically identified DENV transmission chains across the metropolitan area. We used such estimates of transmission to empirically evaluate the epidemiological impact of targeted indoor residual spraying (TIRS) with insecticides (application of residual insecticides at *Ae. aegypti* indoor resting sites). The city of Cairns was identified as a central hub for DENV transmission (95.2% of transmission events of Cairns residents and 60.4% of transmission events of residents of satellite towns were tracked to locations found within Cairns). Out-of-home exposure accounted for 57.2% of all putative transmission sites. Performing IRS in contact locations lead to a significant protective efficacy (0.86-0.96) in preventing DENV transmission. We provide quantitative evidence of the positive value of enhancing surveillance of urban DENV by performing location-based contact tracing and targeting indoor residual spraying operations at putative transmission sites identified from such data. While this approach was applied in a developed urban center, the potential for its implementation in DENV endemic areas will need to be evaluated.

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ZIKA VIRUS IN THE AMERICAS: A MODEL-BASED ASSESSMENT OF FACTORS AFFECTING EMERGENCE

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Zika virus is a mosquito-borne pathogen that has emerged as a major threat to human health as the virus continues to spread throughout Latin America. Zika virus has now been linked to severe birth defects, as well

as an increase in Guillain-Barré syndrome. The parameters that affect transmission of the virus between mosquitoes and humans are still poorly understood beyond extrapolations from similar viruses. It is known that the virus is transmitted by mosquitoes in the genus *Aedes* (*Ae. aegypti* and *Ae. albopictus*), two invasive species that are both well-established within the United States. These species are closely associated with human habitation and breed in a diverse array of peridomestic containers that hold standing water. We have developed a deterministic model accounting for spatio-temperal heterogeneities in temperature throughout the United States to predict seasonal limits and peaks of Zika virus infection in order to focus vector control efforts and anticipate potential diagnostic testing demands. The model is informed by emerging field and laboratory data, including vector studies from our laboratory, and we contrast our Zika estimates for the U.S. to those for a dengue-endemic area in Iquitos, Peru to understand whether housing characteristics such as the availability of window screening and air conditioning in the U.S., are adequate to prevent Zika outbreaks.

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WIND-ASSISTED LONG-DISTANCE MIGRATION OF MALARIA MOSQUITOES IN THE SAHEL

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Over the past decade, malaria control efforts have greatly reduced its burden even in its home base - Africa, raising hopes for malaria eradication in some reader's lifetime. Malaria transmission in Africa extends from the Equatorial Zone to the Sahel where it is confined to the short rainy season. Persistence of anopheline vectors in areas without surface waters for 3-8 months a year attests for the sophisticated strategies employed by the vectors. Recent evidence suggests that *Anopheles coluzzii* persists locally throughout the dry season by a form of diapause (aestivation), whereas *A. gambiae* and *A. arabiensis* rely on long-distance migration from areas where reproduction continues year round. This contradicts the widely accepted view that these vectors do not disperse beyond a few kilometers in a lifetime. Here, we summarize aerial sampling of insects 100-300 m above ground conducted between May 2012 and November 2015 in four Sahelian villages. A total of 30 *A. gambiae* s.l. and 117 *A. pharoensis* were captured among >3,000 mosquitoes and over half a million other insects during 747 aerial night collections. No mosquitoes were captured in 502 control captures raised briefly to 120 m during launches and retrievals, corroborating that these species were intercepted at high altitudes rather than near the ground. A high proportion of the mosquitoes were gravid, indicating that they might carry human pathogens. Because such movements of mosquito vectors are regular and involve many thousands of mosquitoes per night, they have important implications for disease emergence and reemergence as well as for disease elimination programs. A comprehensive analysis of these data will be presented.

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GENERIC AND STANDARDIZED DATA COLLECTION FORMS AND DATABASE APPLICABLE TO DIVERSE ENTOMOLOGICAL STUDIES OF MOSQUITOES

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Studies of malaria vectors and other vector-bone diseases encompass a remarkably diverse array of designs and rapidly generate large data volumes. Such data is normally collected or recorded using experiment-specific forms that require frequent, error-prone redesign leading to badly or inconsistently structured data, difficulty in sharing or linking. Standardised data schema, databases and even public data repositories exist for genetic data for malaria parasites and for their human and mosquito hosts and similar controlled and standardised systems are available for epidemiological studies of malaria-infected human beings. However, equivalent systems for studies of the mosquitoes which mediate transmission do not exist. We have developed 1) a generic schema, 2) paper-and electronic-based customizable data collection forms, and 3) a database web-based application - to provide structure to data at the point of mosquito data collection and streamline use of databases that consistently link field and laboratory data. The database is built in such a way that it can be linked to other system such demographic surveillance systems and epidemiological based-databases. As a result, data from diverse mosquito studies conformed to a developed generic schema, with data collection forms recording the experimental design, sorting of collections, details of sample pooling or subdivision, and additional observations using standardized formats. The database stores and links data, generates summarized reports, enhances data sharing and dissemination from multiple experiments, projects, and studies. Currently, the user uptake includes 20 experiments, 10 projects, and 20 users at 3 research and control institutes in 3 African countries, resulting in 13 peer-reviewed publications. This vector database is expected to advance vector control research especially for resource-limited tropical settings lacking specialized software or informatics support.

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IMPLEMENTATION OF A MULTI-COUNTRY RESPONSE TO THE EBOLA VIRUS DISEASE EPIDEMIC IN WEST AFRICA: LESSONS FROM THE FIELD

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The recent West African outbreak of Ebola virus disease (EVD) is the largest ever recorded. Starting from September 2014, International Medical Corps (IMC) opened five Ebola Treatment Units (ETUs) in Liberia and Sierra Leone, which cumulatively collected nearly 25,000 pages of epidemiologic, clinical, and laboratory data. To extract this data, each chart was either manually copied across the fence between the high-risk zone and low-risk zone or was imaged in the high-risk zone using a GoPro camera. Data were then entered into separate electronic databases, which were combined into a single relational database. Data quality assurance identified an overall final error rate of 1.2%. The full IMC database includes 2768 patient presentations, of which 2485 were admitted to an ETU, and 2329 had outcome data available. Of these, 1506 (65%) patients were from Sierra Leone while 823 (35%) were from Liberia. 54% of patients were male, and the median age was 30 (IQR 18-43). 10% of admitted patients were < age 5 and 11% were > 55. Among all admitted patients, 461 (20%) tested positive for EVD. 192 recovered to discharge, 5 were transferred to other facilities, and 264 died, for a case fatality ratio (CFR) of 58%. Among EVD negative patients, 154 of 1868 died, for a CFR of 8%. Average length of stay was 14.6 days for EVD positive patients who recovered and 5.6 days for those who died. Although more males were admitted as suspect patients than females, a larger proportion of females were diagnosed as EVD positive (26% vs 15%). EVD positive patients aged 15-24 had the lowest CFR (37%), while patients < 5 and > 55 had the highest CFRs (93% and 70%, respectively). CFRs were also higher in Sierra Leone (61%) than Liberia (53%). While several prior reports have documented the experiences of individual ETUs, this study is the first to present data from multiple ETUs across two countries run by the same organization with similar clinical protocols. Our experience

demonstrates that even in austere settings under difficult conditions, it is possible for humanitarian organizations to collect high-quality clinical and epidemiologic data during a major infectious disease outbreak.

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THE NATURAL HISTORY OF EBOLA VIRUS DISEASE: A RETROSPECTIVE STUDY OF THE WEST AFRICA EPIDEMIC

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This retrospective study documented the natural history of EVD among confirmed patients utilizing data from five Ebola Treatment Units operated by International Medical Corps in Sierra Leone and Liberia in 2014-2105. All patients were treated as per standard treatment protocols based on guidelines developed by the World Health Organization and Medecins Sans Frontieres. Self-reported and observed data on presence of symptoms were collected at admission and during daily rounds by clinical staff. Progression of EVD symptoms were explored using descriptive statistics, differences in mean number of days symptomatic among subgroups were analyzed using t-tests and ANOVA, and survival analyses were conducted utilizing Kaplan-Meier estimators and Cox proportional hazards models. Of 470 confirmed EVD cases treated, 297 met the inclusion criteria of positive EVD diagnosis, known outcome, and reported symptom onset date. To assess progression of symptoms by week, a subset of patients for whom rounding dates were also present were analyzed (n=253). The mean number of symptomatic days at admission was 4.3; this varied significantly by sex (females: 3.9, males: 4.8, $p = 0.036$) and by age category (0-4: 2.4, 5-24: 3.7, 25-44: 4.6, 45+: 5.0, $p = 0.009$). The three most common symptoms by week symptomatic were: Week 1 (n=253) - weakness (67%), anorexia (63%), fever (55%); Week 2 (n=134) - weakness (70%), fever (69%), diarrhea (63%); Week 3 (n=52) - fever (44%), bone/muscle/joint pain (35%), headache (33%). The overall survival rate was 36.9% with no significant difference by sex. All age groups had a lower risk of death when using patients 0-4 years as the reference: 5-24 years, hazard ratio (HR)=0.34 (95% confidence intervals (CI): 0.19-0.59, $p<0.001$); 25-44 years, HR=0.45 (95% CI: 0.27-0.77; $p=0.003$); 45+ years, HR=0.54 (95% CI: 0.31-0.93; $p<0.025$). Among patients who died, mortality occurred in 86% by day 13 of experiencing symptoms. The natural history of EVD among patients in Liberia and Sierra Leone demonstrated consistent progression of nonspecific symptoms and high overall mortality with significantly higher mortality among patients 0-4 years.

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BIAS ADJUSTMENT OF CASE FATALITY RATE ESTIMATES IN THE EBOLA OUTBREAK IN WEST AFRICA

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The recent Ebola outbreak in West Africa caused an officially reported 28,646 cases and 11,323 deaths by 30 March 2016, however, the true burden was likely considerably higher. The case fatality rate (CFR), defined as the proportion of cases that die, is the most important indicator of severity. It is however surprisingly difficult to estimate accurately from data collected routinely during an outbreak. The most reliable estimates in this outbreak were obtained by only considering cases for which the outcome (death or survival) was recorded, however, with only half of the reported cases having an outcome recorded there is substantial scope for biases in this simple estimate if the probability of reporting the outcome depends on the outcome itself. By considering the strong age-dependence of CFR and comparing the proportion of cases with recorded outcome between age groups we assess the differential outcome reporting probability between survivors and fatalities, and adjust CFR estimates accordingly. While in Guinea outcome reporting was near complete for cases entered into the VHF database, we estimated that fatal cases were more likely than survivors by 31% in Liberia and 131% in Sierra Leone to have the outcome reported, leading to an upwards bias in raw CFR estimates. When adjusting for this bias, CFR estimates were corrected from 65.6% (95% CI 63.8 - 67.3%) to 58.8% (51.4 - 65.4%) in Liberia and from 71.2% (69.7% - 72.7%) to 51.7% (45.2 - 61.4%) in Sierra Leone, while estimates in Guinea remained ad 59.2% (57.1 - 61.4%). CFR estimates adjusted for differential reporting were therefore more consistent between countries. Accurate estimates of disease severity are crucially important for public health planning, but are challenging to obtain from data collected typically during an outbreak. The bias of differential outcome reporting is likely a problem in many settings, and the method developed here will therefore be useful in future outbreaks of novel pathogens.

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QUANTIFICATION OF THE IMPACT OF SAFE AND DIGNIFIED BURIALS DURING THE 2013-2016 WEST AFRICAN EBOLA VIRUS DISEASE EPIDEMIC

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Over 28,600 people were infected with Ebola virus disease (EVD) and over 11,000 died in the 2013-2015 West African epidemic. The EVD status of individuals who die in the community, outside of Ebola Treatment Centers, is unknown at the time of death however burial of EVD infected individuals poses a serious risk for continued transmission. Consequently an important component in EVD response is safely burying infected individuals. This pillar of the response was led by the International Federation of the Red Cross and Red Crescent Societies, who buried 47,505 individuals, 2,205 of which were EVD positive. Here we quantify the impact of the Red Cross Safe and Dignified Burial Program (SDB) on the EVD epidemic. Epidemiological and anthropological investigations were completed in communities in Sierra Leone, Guinea and Liberia that had carried out unsafe burials. Forty-five unsafe burials were investigated and 310 contacts identified. Approximately 7 individuals per unsafe burial were reported to have had contact with the index case (IC) and 1.8 infected secondary cases were generated, although this varied by district (range: 0.6-5.5). Contact with the IC during their acute illness and post-mortem was reported for 46% of contacts. Contact with fluids of the IC was the most strongly predictive of transmission followed by physical contact with the IC during their acute illness. Those having contact with the IC before death were 2.5 - 6 times more likely to be infected with EVD, relative to those with post-mortem contact only. By averting 1,477 to 10,452 secondary EVD cases, SDB reduced the size of the epidemic by 5.2 to 36.5%. Through this study it is impossible to ascertain, for those individuals who had contact with the index case before and after death, the exposure that caused their infection. Nevertheless, these results underline the importance of isolating individuals infected with EVD early in order to further limit community transmission. We also quantify for the first time, the importance of SDB as a fundamental EVD control measure and provide an estimate the number of additional infections averted by SDB.

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MORTALITY OUTCOMES AMONG PATIENTS WITH VARIABLE INFECTION STATES WITH EBOLA VIRUS DISEASE AND MALARIA IN SIERRA LEONE: A RETROSPECTIVE COHORT STUDY

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This retrospective cohort study investigated the relationship between infection with Ebola Virus Disease (EVD) and/or malaria and case fatality ratio (CFR) among patients admitted to three Ebola Treatment Units (ETU) in Sierra Leone between December 2014 and September 2015. Standardized care with artemisinin-based combination therapy for empiric malaria treatment was provided to all patients based on International Medical Corps protocols. The cohort was stratified by infection status as: malaria negative/EVD negative (m-/e-), malaria positive/EVD negative (m+/e-), malaria negative/EVD positive (m-/e+) and malaria positive/EVD positive (m+/e+). Mortality outcomes were explored using descriptive statistics and Cox proportional models. Survival regression analyses adjusted for age, and the m-/e- group was set as the baseline comparator. Mortality time of event was derived from chart documentation and all survivors were censored at 28 days from admission. Among 1548 cases treated, 753 met inclusion criteria. The cohort cases included 431 m-/e-, 180 m+/e-, 108 m-/e+ and 34 m+/e+. In the m-/e- group, the CFR was 11%. For m+/e- cases the CFR was 5%, with an adjusted hazard ratios (aHR) of 0.5 (95% CI: 0.3-1.0, p=0.07). Cases found to be m-/e+ had 53% mortality and a greater than five-fold increased risk of death (aHR=5.7, 95% CI: 3.9-8.5, p<0.001). The m+/e+ group had the highest CFR, at 59%, with an aHR of 7.7 (95% CI: 4.5-13.1, p<0.001). Patients admitted to ETUs with confirmed EVD had the highest mortality, and concomitant infection with malaria increased the risk of death in the studied population. Among patients without EVD who were admitted to ETUs, risk of death was nearly twice as high in those without malaria infection as opposed to those with malaria infection, likely due to empiric treatment of all patients with antimalarial medications.

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IMPLEMENTING SEASONAL MALARIA CHEMOPREVENTION (SMC) IN THE CONTEXT OF EBOLA VIRUS DISEASE (EVD) IN GUINEA

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An overlap of symptoms of the EVD and malaria in Guinea created a context for integrated case management of the two diseases, as untreated malaria cases were linked to increased malaria mortality and fever cases (a shared symptom for both diseases) which was deemed to impede the EVD response. SMC began in Guinea during the onset of EVD and in the context of severely diminished demand for health services; EVD was the estimated cause of approximately 74,000 fewer malaria cases seen at health facilities in 2014. The NMCP used EVD case management structures and implemented SMC through a house-to-house strategy in six SMC-eligible districts targeting 210,000 children 3-59 months. A Health Service Delivery Coverage exercise designed to examine perceptions of service delivery acceptability (including SMC) was conducted through focus group discussions and key informant interviews in each of the SMC Districts. It engaged caregivers, local leaders and CHWs who observed a flattening in demand for health services throughout Guinea in reaction to EVD - contributing to the 46,968 confirmed cases of malaria and 86 deaths within the under-five target population in 2014. The total cases of confirmed malaria in the areas that received SMC in 2015 decreased by 29% and by 26% for malaria deaths among the under-five population. Whereas in the two SMC-eligible Districts of Siguiri and Madiana where SMC was not implemented in 2015 there were increases in malaria cases in the under-five population from 2014 to 2015. In Siguiri there was an increase in confirmed malaria cases by 92% (3,352 cases in 2014 and 6900 cases in 2015) and an increase in malaria deaths 7,350% (4 deaths in 2014 and 298 deaths in 2015); while in Madiana there was an increase in confirmed malaria cases by 2,450% (740 confirmed cases in 2014 and 19,633 confirmed cases in 2015) and no increase in malaria deaths. SMC in Guinea, reached 100% of eligible children: demonstrating that even in the context of EVD or potentially other outbreaks, SMC can be delivered at scale and save lives. In 2016, SMC will be scaled up to cover Siguiri and Madiana and the NMCP will continue SMC implementation in all eligible areas in 2017 and beyond.

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EFFECTS OF FUNCTIONAL LATRINE DENSITY ON HOUSEHOLD DRINKING WATER CONTAMINATION, SOIL-TRANSMITTED HELMINTH INFECTION AND DIARRHEA: A SPATIAL ANALYSIS

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India accounts for 60% of the 2.4 billion people practicing open defecation worldwide. A large cluster-randomized trial (CRT) in Orissa found no village-level health impacts of the Government of India's Total Sanitation Campaign. Village-level coverage varied greatly; latrines went unused, often due to poor construction/functionality. Using geospatial data from households in 50 intervention villages of the CRT in Orissa, we assessed environmental health impacts of *functional* latrine density on a fine spatial scale. Coverage of all latrines and functional latrines within 25m, 50m, 100m, and 200m of households was calculated via a multiple ring buffer analysis in ArcGIS. Outcomes were household drinking water contamination (N=1009), soil-transmitted helminth (STH) infection (N=822), diarrhea among all ages (N=1275) and children <5 (N=1017). Multivariate regressions adjusted for village-level clustering with Generalized Estimating Equations. Covariates included location of water source, household population, and whether or not a household, itself, owned a functional latrine. Increased latrine coverage in 200m was associated with decreased levels of thermotolerant coliform (TTC) in household drinking water (β=-5.05, 95% CI -9.81, -0.29). Each additional functional latrine in 25m was associated with a decrease of 28.9 cfu TTC per 100 mL (CI -57.5, 9.8). Odds of STH infection decreased by 10% for each additional latrine, regardless of functionality, in 25m (β=0.903, CI 0.819, 0.994). For every 10 additional latrines in 25m, regardless of functionality, household longitudinal diarrhea prevalence (all ages) *increased* by 2.13 days per 1,000 person days (CI 0.06, 4.2). A 10% increase in functional latrines in 25m was associated with 1.4 fewer days of diarrhea per 1,000 person days (CI -5.5, -0.1). Households owning functional latrines, themselves, had 8 fewer days of childhood diarrhea per 1,000 person days (p<0.05). Ensuring 100% sanitation coverage and functionality within the immediate surroundings of the home is critical for reducing exposure to pathogenic feces that cause diarrheal diseases.

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FECAL CONTAMINATION ALONG MULTIPLE ENVIRONMENTAL PATHWAYS IS ASSOCIATED WITH SUBSEQUENT DIARRHEA AMONG CHILDREN IN RURAL BANGLADESH

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Pathogens associated with diarrhea are transmitted from feces to new hosts through multiple environmental pathways: drinking water, ambient water, hands, soil, food, and flies. Understanding the relative risks of exposure to fecal contamination along each pathway could be valuable in the design of interventions to interrupt transmission and reduce diarrhea. We conducted a prospective study among 1843 households in rural Bangladesh to quantify levels of fecal contamination along multiple environmental pathways and assess their association with subsequent risk of under-five child diarrhea. Measurements were collected during two sequential visits to each study household. During the first visit, sampling at each household included: child hand rinse, stored drinking water, source drinking water (tubewells), pond water, soil from the child's play area, food served to young children and flies captured from the food preparation area. All samples were analyzed for fecal indicator bacteria (most probable number [MPN] of *E. coli* and fecal coliforms) using the IDEXX Colilert-18 Quanti-Tray system. During the second visit (conducted 4-10 days after the initial visit to encompass typical incubation periods for gastrointestinal pathogens), interviewers collected caregiver-reported child gastrointestinal symptoms. We used generalized linear models with robust standard errors to estimate the relationship between the presence and concentration of fecal indicator bacteria and subsequent diarrhea prevalence. Child diarrhea prevalence following the field team's previous visit to the household increased by 31% with *E. coli* presence on child hands (PR=1.31, 1.06-1.63) and 17% for each log10 increase in fecal coliform counts in soil (PR=1.17, 1.04-1.32). Our findings suggest that child hands and household soil are important transmission pathways for diarrheal illness among children under five in rural Bangladesh.

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QUANTIFYING FECAL CONTAMINATION LEVELS OF DRINKING AND AMBIENT WATERS, HANDS, FOOD, SOIL AND FLIES IN THE DOMESTIC ENVIRONMENT IN RURAL BANGLADESH

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Fecal-oral pathogens are transmitted from feces to hosts through a variety of environmentally mediated pathways. Characterizing levels of fecal contamination along these pathways can inform the development of targeted interventions to reduce fecal exposures. We quantified fecal indicator bacteria along different transmission routes in the domestic environment in 1843 households in rural Bangladesh. We collected samples of source (tubewell) and stored drinking water, pond water, child hand rinse, weaning food, flies caught in the food preparation area and soil collected from children's outdoor play area. We analyzed samples for *E. coli* with the IDEXX most probable number (MPN) method and calculated the geometric mean of *E. coli* concentration for each type of sample. 24% of tubewells and 58% of stored water samples were contaminated with *E. coli*. A typical pond water sample contained almost 4-log MPN *E. coli* per 100 mL. 40% of children had *E. coli* on their hands; children ≥ six months old had 0.3-log MPN per two hands more *E. coli* than children < six months of age (p<0.001). A typical soil sample had approximately 5-log MPN *E. coli* per dry gram. Soil in the vicinity of human or animal feces had 0.2-log MPN higher *E. coli* than soil collected from areas with no feces (p<0.001). Soil from sunlit areas had 0.2-log MPN fewer *E. coli* than soil from shaded areas (p<0.001), and levels of *E. coli* were positively correlated with the moisture content of samples (p<0.001). 59% of stored food samples contained *E. coli*; food stored in a covered container had 0.2-log MPN fewer *E. coli* than food from uncovered or partially covered containers (p=0.02). 54% of captured flies had *E. coli* contamination; a typical fly had approximately 3-log MPN *E. coli.* Fecal indicator bacteria levels were higher among all seven pathways in the rainy season (p≤0.01). Our findings demonstrate ubiquitous fecal contamination along multiple pathways in rural Bangladeshi households and highlight the occurrence of high levels of fecal indicator bacteria in ponds and especially courtyard soil in this setting, drawing attention to these understudied pathways for diarrheal disease transmission.

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UNSAFE CHILD FECES DISPOSAL IS ASSOCIATED WITH ENVIRONMENTAL ENTEROPATHY AND IMPAIRED GROWTH

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This study was undertaken to investigate the relationship between unsafe child feces disposal, environmental enteropathy (EE), and impaired growth, we conducted a prospective cohort study of 216 young children in rural Bangladesh. Unsafe child feces disposal, using the WHO/ UNICEF Joint Monitoring Program definition, was assessed using 5 hour structured observation and caregiver reports. Anthropometric measurements were collected at baseline and at a nine month follow-up. Stool was analyzed for fecal markers of EE: alpha-1-antitrypsin, myeloperoxidase, neopterin (combined to form an EE disease activity score), and calprotectin. Eighty four percent of households had an unsafe child feces disposal event during structured observation and 75% had caregiver reported events. There was no significant difference in observed unsafe child feces disposal events for households with or without an improved sanitation option (82% vs. 85%, p=0.72) or by child age (p=0.96). Children in households where caregivers reported unsafe child feces disposal had significantly higher EE scores (0.82 point difference, 95% confidence interval (CI): 0.11, 1.53), and significantly higher odds of being wasted (Weight for Height z-score (WHZ) <-2 SDs) (9% vs. 0%, p= 0.024). In addition, children in households with observed unsafe feces disposal during structured observation had a significantly reduced change in Weight for Age z-score (-0.34 (95% CI: -0.68, -0.01) and WHZ (-0.52 (95% CI: -0.98, -0.06)). In conclusion, unsafe child feces disposal was significantly associated with EE and impaired growth in a pediatric population in rural Bangladesh. Interventions are needed to reduce this high risk behavior to protect the health of susceptible pediatric populations.

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THE MAPUTO SANITATION (MAPSAN) TRIAL: ASSESSING A SANITATION INTERVENTION'S IMPACT ON HELMINTHIASIS IN CHILDREN <5 YEARS OLD IN MAPUTO, MOZAMBIQUE

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The MapSan study, in Maputo, Mozambique, is a first of its kind, controlled before and after trial of the impact of a shared sanitation intervention (pour-flush latrines) on soil-transmitted helminth (STH) and enteric infections in children <5 years. Despite progress in urban sanitation coverage, residents of urbanizing, unplanned communities in large cities of Sub-Saharan Africa experience elevated disease risks associated with poor sanitation. Dense urban environments are critical settings for targeted sanitation improvements since the risks of unsafe excreta disposal can be much greater within a dense urban population compared with a low-density rural population. For the study, stools were collected from children in intervention and matched-control arms to assess baseline STH prevalence prior to intervention exposure. Stools were analyzed by Kato Katz (N=671) to quantify several STHs (including *Trichiura trichuris*, *Ascaris lumbricoides*, and hookworm) and will be shipped to the US for multiplex-STH qPCR analysis. Overall baseline prevalence for any STH infection by Kato-Katz is 46% and is similar between study arms. As expected, prevalence increases with age [in years (OR 1.96, CI: 1.67, 2.31)]. T. *trichuris* and A. *lumbricoides* are the most commonly observed STHs with prevalences of 23% and 38%, respectively. Coinfection with multiple STH was observed in 16% of samples. Post-exposure sample collection is ongoing and data will be available starting in Summer 2016. The most recent nationwide survey of STH in school-aged Mozambican children (2005-2007) found a combined prevalence of 53.5%. Our baseline results provide a more complete picture of the STH burden in Mozambique by reporting on younger children who are often missed in school-based prevalence surveys. Our pending endline results will add evidence to the conversation of how to best serve the approximately 2.5 billion people who currently lack access to safe sanitation.

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WATER, SANITATION AND HYGIENE (WASH) AND ENVIRONMENTAL RISK FACTORS FOR SOIL-TRANSMITTED HELMINTH INTENSITY OF INFECTION IN TIMOR-LESTE, USING REAL TIME PCR

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No investigations have been undertaken of risk factors for intensity of soil-transmitted helminth (STH) infection in Timor-Leste. We present the first analysis of combined water sanitation and hygiene (WASH), environmental, and socioeconomic risk factors undertaken using intensity of infection classes developed from qPCR diagnosis of STH. Questionnaires were used to collect WASH and demographic data from 24 villages in Manufahi District, Timor-Leste. An algorithm was developed to correlate PCR cycle threshold values to eggs per gram of faeces equivalents, using seeding experiments. Open-access environmental variables were obtained. A socioeconomic quintile was developed using principal component analysis. Multinomial mixed-effects regression was used to assess risk factors for intensity of Necator americanus and Ascaris infection in 2152 participants. In adjusted models incorporating WASH, socioeconomic and environmental variables, environmental variables were generally associated with infection intensity for both N. americanus and Ascaris spp. Precipitation (in centimetres) was associated with increased risk of moderate-intensity (adjusted relative risk (ARR) 6.1; 95% confidence interval (CI) 1.9-19.3) and heavy-intensity (ARR 6.6; 95%CI 3.1-14.1) N. americanus infection, as was sandy-loam soil around household (moderate-intensity ARR 2.1; 95%CI 1.0-4.3; heavy-intensity ARR 2.7; 95%CI 1.6-4.5; compared to no infection). For Ascaris, alkaline soil around the household was associated with reduced risk of moderate-intensity infection (ARR 0.21; 95%CI 0.09-0.51), and heavy-intensity infection (ARR 0.04; 95%CI 0.01-0.25). Few WASH risk factors were significant. Our novel approach of assigning infection intensity classes to PCR-diagnosed STH infection requires further research. In this high-prevalence setting, significant risk associations with environmental factors suggest that anthelmintic treatment should be integrated with other interventions, as conditions are favourable for ongoing environmental transmission. Integrated STH control strategies should be explored as a priority.

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WASH FOR WORMS: END-POINT RESULTS FROM A CLUSTER RANDOMIZED CONTROLLED TRIAL OF THE IMPACT OF A COMMUNITY-BASED INTEGRATED WASH AND DEWORMING PROGRAM ON SOIL-TRANSMITTED HELMINTH INFECTIONS

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Soil-transmitted helminth (STH) infect an estimated 1.45 billion people worldwide, therefore remaining a global health problem. Deworming programmes are effective in reducing STH prevalence but rapid reinfection occurs in the absence of decreased environmental contamination. Therefore WASH interventions are obvious candidates for sustainable control. WASH for WORMS is a cluster-randomised controlled trial to quantify the impact of a community-based WASH intervention integrated with periodic mass distribution of albendazole on infections with STH and protozoa, compared to mass deworming alone. In this trial, initiated in 2012, all participating communities in Timor-Leste received mass deworming every 6 months (for 2 years) and half of them also received the WASH intervention. The WASH intervention was implemented by WaterAid, Australia and included promotion of household latrines (based on "Community Led Total sanitation"), improved access to water and hygiene promotion. Infection prevalence and intensity were measured by qPCR. At baseline, the prevalence of STH in the 24 villages was high, with 62.3% of the participants infected with *Necator americanus*. In the intervention arm, *N. americanus* decreased from 62.8% to 32.2% at the 1st follow-up (FU1), with a further decrease to 21.7% at the 2nd follow-up (FU2), one year after completion of the WASH intervention. In the control group, *N. americanus* decreased from 61.8% to 36.9% at FU1, reaching 20.5% at FU2. At this time point, 77.7% of households in the intervention arm had a latrine whereas in the control arm 20.5% of the houses had one. Participating villages were followed for an additional year, with data collection ending in April 2016. Results will be presented for the study end-points and discussed in the context of the uptake of the WASH intervention. This trial is the first reported RCT evaluating the impact of integrated WASH and deworming interventions on STH infection; and will provide essential evidence for optimizing integrated STH control programmes.

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THE NEW WORLD HEALTH ORGANIZATION APPROACH TO SURVEILLANCE FOR TRACHOMA, EXPERIENCE IN NEPAL AND ADDED BENEFIT OF ANTIBODY PREVALENCE TO *CHLAMYDIA TRACHOMATIS* PGP3 PROTEIN: NESTS STUDY

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As part of the global effort to eliminate trachoma, the leading cause of preventable infectious blindness in the world, the World Health Organization (WHO) requires a second survey for trachoma at least two years after districts have stopped mass drug administration, to determine if re-emergence has occurred. Useful markers, like antibodies to chlamydial antigens, and chlamydial DNA testing for elimination of trachoma, are being investigated for utility in these surveillance situations. Using the new WHO guidelines, this study was undertaken to conduct surveillance surveys and to evaluate the possible role of tools in surveillance programs in 4 districts in Nepal. 15 randomly selected clusters within four districts were chosen. In each cluster, 50 randomly selected children ages 1-9 year olds and 100 adults ≥15 years old were examined for TF±TI and TT respectively. Eye swabs were taken from all children to test for *C. trachomatis* (CT) infection using the Cepheid GeneXpert platform. Dried blood spots were collected from children to determine antibody positivity to the *C. trachomatis* antigen pgp3. Blood spots were processed on the Luminex-100 platform following standard procedures. Data were analyzed as simple frequencies, and age stratified proportions. Results: Districts were 2, 4, 8, and 10 years from last program activities. In the sampled 4,042 children, only 11 TF cases and 3 CT infection were found. Overall antibody positivity was found in 1.9% of samples with no increase in frequency by age. There was no evidence for clustering of antibody positivity by community. Once adjusting for TT already known to the health system, rate was <1/1,000 population in all districts. In conclusion, no evidence of re-emergence of trachoma was found in four districts in Nepal during surveillance surveys as late as 10 years after cessation of all program activities. The absence of an increase in age seroprevalence suggests this tool may measure interruption of transmission of *C. trachomatis*. This study provides an even stronger empirical basis for the new WHO guidelines for surveillance of trachoma, and adds new knowledge on surveillance for trichiasis.

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SERO-SURVEILLANCE IS AN INFORMATIVE INDICATOR OF TRACHOMA TRANSMISSION INTENSITY

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Trachoma remains the world's leading infectious cause of blindness. The World Health Organization (WHO) has a target of elimination of trachoma as a public health problem by 2020. Validation of elimination of trachoma requires that the clinical sign trachomatous inflammation—follicular (TF) be less than 5% in children aged 1-9 years old. However, in settings where transmission is low, grading TF can be challenging, as other bacterial infections and dust may aggravate the eyelid, producing TF-like inflammation that may not be associated with ocular chlamydia infection. Serology has been successfully used as an indicator of transmission intensity for a number of pathogens, and recent work has evaluated the potential for antibody-based testing in children for trachoma surveillance. We used serological data collected from a range of trachoma transmission settings—including high (TF greater than 30%), medium (TF 10-30%), and low (TF less than 10%) prevalence settings with ongoing transmission in Tanzania as well as post-elimination validation surveys in Nepal—to estimate the sero-conversion and reversion rates with a sero-catalytic model for all sites for which appropriate data was available. We then developed a mixed effects regression model to assess which key epidemiological variables (age, gender, number of known past rounds of antibiotic treatment) across multiple surveillance sites were significant predictors of antibody titre, allowing them to vary by trial site. We found that TF prevalence and age are good predictors of antibody titre within the community. Our findings demonstrate that prevalence of antibodies against chlamydial antigens may be informative indicators of transmission intensity and that serological surveillance could be a valuable approach for-post-elimination validation surveys.

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INFLUENCE OF INDIVIDUAL AND ENVIRONMENTAL FACTORS ON THE PREVALENCE OF TRACHOMA IN THE HEALTH DISTRICT OF MOKOLO AFTERTHREE YEARS OF MASS TREATMENT WITH ZITHROMAX AND TETRACYCLINE

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It is commonly believed that personal hygiene and the state of cleanliness of the environment are significant factors in the spread of trachoma. The strategy for control is through SAFE (Surgery, Antibiotic treatment, Facial cleanliness and Environmental improvement). The health district of Mokolo in the Far-North Region of Cameroon, recognized as endemic with trachoma in 2010, benefited from the azithromycin and tetracycline mass distribution during three consecutive years, supported by HKI with funding from USAID's ENVISION Project, managed by RTI International. Rarely actions in F and E components were registered. We aimed at evaluating the influence of facial cleanliness and the environmental factors on the residual infection of trachoma after the treatment. We carried out a descriptive cross-sectional study based on a stratified random sampling in Mokolo in 2015. Selected were 20 communities representing clusters; with 25 households in each. After an interview with the households and ophthalmic assessments by trained trachoma graders, data were collected using the numerical tablets transferred to a central base, before being analyzed using Software SPSS. Among the 827 children aged 1 to 9 years examined, the prevalence of active trachoma (TF&TI) was 1.7% in 2015 against 18.1% in 2010. The proportion of children having dirty faces was 14.85%. A strong association was found between the facial uncleanliness and active trachoma infections (OR = 14.79; p<0.01). Out of 91.6% of houses with cattle, 94.7% cohabited with the animals inside the homes. However 60.8% of the households were located within less than 30 min from a source of water. Despite drastic reduction of the disease prevalence to the threshold of the stopping MDA, the individual and environmental factors remain a strong influence. This could compromise the sustainable elimination of trachoma in this health district. More efforts on the F and E components of the SAFE strategy is needed.

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A SPATIAL ANALYSIS OF ENVIRONMENTAL FACTORS AND TRACHOMATOUS-INFLAMMATION FOLLICULAR AMONG CHILDREN 1-9 YEARS IN SOUTH GONDAR, ETHIOPIA

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South Gondar zone in the Amhara region of Ethiopia has a high prevalence of trachomatous-inflammation follicular (TF), a clinical sign of trachoma, among children ages 1-9 (25.9%). This may not be entirely explained by individual behaviors such as facial cleanliness or household factors like latrine access. Environmental covariates like rainfall, altitude, vegetation, and temperature may impact TF prevalence but there are few published studies assessing the spatial relationships of environmental factors and TF clustering in a hyperendemic setting. Using a multi-stage, cluster randomized design, 12,108 households were surveyed in 313 villages in 12 districts in South Gondar in 2011. We assessed TF prevalence in all children 1-9 years in each household. TF prevalence was aggregated by village for those with valid GPS coordinates. A point pattern analysis of 313 villages was performed using weighted K functions for global clustering and a Getis G* for local clustering (|z|>3.71). Altitude, global precipitation measurement (GPM), naturalized difference vegetation index (NDVI), and land surface day temperature raster images were overlaid with TF clusters. The weighted K function did not identify aggregation up to 100km. Local Getis G* tests identified clustering starting at 10km. Hot spots, areas where high prevalence villages tend to occur near each other, were identified in northern districts of Ebinat and Libo Kemkem. Cold spots, areas where low prevalence villages occur near each another, were identified in central districts of Debre Bahir Town, Lay Gayent, and Estie. Preliminary descriptive analyses suggest that cold spots may be associated with higher elevations and NDVI. Hot spots may be associated with lower elevations and NDVI. Subsequent analyses will include descriptive mapping of select environmental factors and multivariate logistic regressions to identify environmental factors associated with TF clustering. Risk maps will be generated with the associated environmental factors to identify areas of heightened risk which may help inform trachoma control activities in these areas.

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USE OF MULTIPLE STRATEGIES TO MOBILIZE TRACHOMATOUS TRICHIASIS CASES FOR SURGERY IN 4 DISTRICTS OF KATSINA STATE, NIGERIA

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Trachomatous Trichiasis (TT) is blinding stage of trachoma and could cause blindness if not treated. To prevent the blindness, surgical intervention is required. In Katsina State, Nigeria, the ultimate intervention goal (UIG) for TT surgery in 8 Local Government Areas (LGAs) is 15,760. With the support of the Queen Elizabeth Diamond Jubilee Trust, 19 ophthalmic nurses were trained to provide surgeries using the bilateral tarsal rotation (BTR) method. A single TT case mobilization strategy was recommended and initially implemented, i.e. by trained TT case finders to identify and refer cases for surgery. There was a low turn-out of TT cases. To maximize the productivity of surgery, different methods were then tested in order to mobilize patients to access the free service. Barriers to access, such as fear of surgery, distance, poverty level of the affected people and gender barrier, informed the decision to adopt context specific case mobilization method in 4 selected LGAs. TT surgery camps were organized in Daura emirate comprising of Zango, Daura, Mai'adua and Baure LGAs between October and December 2015. A combined strategy of mobilization of TT patients was used in the weeks preceding the outreaches. These included radio announcements, town announcers, announcements in public places such as mosques and market places, in addition to trained TT case finders. With combined mobilization strategy, there was a resultant spike in the productivity as well as the frequency of outreaches being conducted. Two surgery camps were conducted in October and December which resulted in high turn-out of TT cases for surgery. Thus 7 outreaches were conducted within December alone, yielding 245 persons operated in 293 eyes. This caused an increase in productivity as compared to previous months when only the case finder method was used. The number of persons presenting themselves for surgery more than doubled during the same period. Regular outreaches are very important for TT surgery. However, multiple mobilization strategies employed for outreach activities are required in order to reach the UIG and achieve the year 2020 trachoma elimination goal in Nigeria.

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CHLAMYDIA TRACHOMATIS INFECTION IN AMHARA, ETHIOPIA 2011-2015

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Trachoma is caused by the bacteria *Chlamydia trachomatis* (Ct). Infection data, collected in the programmatic setting, would help in understanding the impact of mass drug administration (MDA) interventions as well as the relationship between the clinical signs of trachoma and infection. This study aimed to understand the effect of multiple years of MDA on Ct infection in the Amhara region, Ethiopia by describing the prevalence of Ct infection in a representative sample of children ages 1-5 years. Population-based trachoma impact surveys were conducted in all districts of Amhara, from 2011 to 2015, following 5 years of SAFE interventions. Ocular specimens were collected from randomly selected children ages 1-5 years whose households were included in the surveys to estimate the zonal prevalence of Ct infection. Samples from each district were pooled and the Abbott Realtime PCR assay was used to detect Ct DNA using the m2000 system. District prevalence was determined from the district pooled prevalence using maximum likelihood techniques. Zonal prevalence and confidence intervals (CI) were estimated using survey procedures in Stata. A total of 15,636 samples were collected across 10 zones of Amhara. The prevalence of trachomatous-inflammation follicular (TF) in children ages 1-9 years in Amhara region was 26.1%, (95%CI: 25.1, 27.1), zonal range: 13.6% to 54.6%, and the regional prevalence of trachomatous-inflammation intense (TI) was 5.6% (95%CI: 5.2, 6.0), zonal range: 3.4% to 13.6%. The prevalence of Ct infection in children ages 1-5 years was 5.5% (95%CI: 4.2, 6.7), with zonal estimates ranging from 1.0% in Awi zone to 15.3% in Waghemra. Ct infection and TI were very highly correlated at the zonal level (Spearman correlation(r)= .92; P=0.0002), while Ct infection and TF were moderately correlated (r= .57; P=0.084). To our knowledge, this is the first report of Ct infection data at a regional level within a programmatic setting. Despite over 5 years of MDA, a considerable amount of Ct infection remains in Amhara. TI was highly correlated with Ct infection and may represent a potential marker of infection that programs could use in measuring impact.

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TRACHOMA IMPACT SURVEYS IN MAINLAND TANZANIA: LESSONS LEARNED FROM IMPLEMENTATION OF THE SAFE STRATEGY

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Tanzania started implementation of the surgery, antibiotics, facial cleanliness and environmental change (SAFE) strategy for trachoma control in 1999. Tanzania has made substantial progress towards meeting Global Elimination of blinding Trachoma by the year 2020 (GET2020) objectives. By the end of 2015, 37 out of 55 districts under SAFE implementation had achieved the goal for stopping mass drug administration (MDA) of trachomatous inflammation-follicular (TF) prevalence <5% in children aged 1-9 years. We investigated the trends of TF prevalence and annual MDA coverage. Data from 13 districts were analyzed: 2 had TF<5% (Handeni and Kwimba); 2 had TF>10% after 1st impact survey (Kilindi and Kongwa); 2 had TF=5-9.9% after 1st impact survey (Manyoni and Mpwapwa); 3 had stopped MDA due to programmatic challenges (Monduli, Longido and Ngorongoro); and 4 had baseline TF=5-9.9% and no MDA (Morogoro Rural, Mvomero, Singida Urban and Rombo). Time trends of TF prevalence and MDA coverage were plotted. In 2 districts with TF<5% at 1st impact survey, Handeni had 3 consecutive years of MDAs while Kwimba had 1 year of MDA. In 2 districts with TF>10% at 1st impact survey, Kilindi had no change in TF prevalence despite 4 MDA rounds of varying coverage while in Kongwa, TF was >10% despite 14 MDA rounds of varying coverage. Among districts with TF=5-9.9% (at the 1st impact survey), Manyoni had no change in prevalence (at the 2nd impact survey) despite a high coverage MDA; while Mpwapwa had TF<5% after a high coverage MDA. MDA implementation was delayed in Monduli, Longido and Ngorongoro, then implemented with low coverage in Monduli and Longido, and high coverage in Ngorongoro but stopped in all 3 districts due programmatic challenges. In 4 districts where baseline TF=5-9.9%, follow-up surveys after 10 years showed that TF was <5% in absence of interventions. The reduction of TF varied by district. Results suggest that low MDA coverage may contribute to the reduced impact on TF decline. To achieve GET 2020 objectives: MDA needs to be started promptly and sustained to achieve maximum impact; high MDA coverage needs to be achieved; and prompt impact surveys and action are needed.

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COMPARISON OF EPIDEMIOLOGY, TRANSMISSION DYNAMICS AND CLINICAL PRESENTATIONS OF GENITAL AND SKIN ULCERATIONS CAUSED BY *HAEMOPHILUS DUCREYI*: EXPERIENCE FROM HYPER-ENDEMIC AREAS IN AFRICA AND PACIFIC ISLANDS

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Haemophilus ducreyi (HD), a fastidious gram-negative bacterium, has been known to cause the sexually transmitted genital ulcerations (i.e. Chancroid) in tropical countries and hyper-endemic situations were reported in Southern Africa during the 1990s. Recently, HD has been found to be associated with cutaneous ulcerations among children under 15 years old in yaws endemic areas. A retrospective case review was conducted on 117 laboratory-confirmed HD genital ulcer cases (genital HD) that presented to the Sexually Transmitted Disease (STD) clinic in Johannesburg, South Africa and 60 laboratory-confirmed cutaneous ulcerations (non-genital HD) in children seen during the community-based surveillance activities in Vanuatu. The demographic, clinical presentations (including photographs of lesions) and laboratory findings of two patient population were compared. The laboratory investigations include syphilis serology using RPR and TPPA; multiplex real-time PCRs for HD, herpes simplex virus (HSV), Treponema pallidum subspecies (TP), and Mycobacterium ulcerans (buruli ulcer). In addition, culture for HSV and HD, and HIV serology were performed on samples from the genital-HD group. The mean ages of patients with genital and non-genital HD lesions were 33 years (± 9) and 8 years (± 3), respectively. The majority of patients in both groups presented with large, multiple, punched-out ulcerations with soft edges and purulent bases which are clinically indistinguishable between genital and non-genital HD lesions. Purulent inguinal lymphadenopathy (i.e. Bubo) was observed in 38 (32.5%) genital HD cases compared to none in non-genital HD patients. RPR seropositivity was 15.5% in genital HD group and 35.5% in non-genital HD group. In conclusion, clinicians should be aware of the presence of non-sexual transmission of HD and TP in some geographical areas and carefully consider the potential etiology of cutaneous genital and non-genital ulcerations in residents from those areas. Laboratory confirmation and differentiation of ulcer etiology at a reference laboratory would be required to support the clinical diagnosis and management.

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DIFFERENCES IN THE CLINICAL AND LABORATORY FEATURES OF ONCHOCERCIASIS IN ENDEMIC AND NONENDEMIC POPULATIONS REFLECT IMMUNE-MEDIATED PROCESSES

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Some filarial infections including *Loa loa* and *Wuchereria bancrofti* may have different presenting features in endemic populations compared with immunologically naïve expatriate hosts. However, few studies have examined this relationship in *Onchocerca volvulus* infection. To study this question more directly, we identified all patients diagnosed with active *Onchocerca volvulus* infection at the National Institutes of Health between 1976 and 2016. Study subjects received a detailed baseline assessment including history, physical examination, ophthalmologic evaluation, and extensive laboratory investigations. Forty returned travellers (TR) and 36 endemic (END) subjects had onchocerciasis acquired almost entirely in West and Central Africa. The TR more frequently reported a pruritic rash and 28% had acute papular dermatitis on examination compared with only 2.8% of END ($p = 0.004$). Skin pigmentation changes, excoriation or lichenification occurred in 47% of the END but in only 18% of TR ($p = 0.007$). Although similar numbers in both groups reported ocular symptoms, documented onchocercal eye involvement occurred in 16.7% of the END and in none of the TR ($p = 0.009$). There were no differences in any other clinical parameters including pruritus, arthralgia, edema, lymphadenopathy, or of the presence of onchocercomata (2 in the TR, 6 in the END; $p = 0.14$). Fourteen (35%) TR and 7 (19%) END patients had microfiladermia ($p = 0.43$). Geometric mean (GM) absolute eosinophil counts were significantly ($p<0.05$) higher in the TR (850/mm3) compared to the END (438/mm3). By contrast, we observed higher serum polyclonal IgE levels in endemic patients (GM = 826.1 IU/mL) compared to the TR (206.3 IU/mL; $p < 0.001$). Parasite-specific IgG4 levels were also significantly higher in END than in TR patients ($p = 0.026$). Although there is substantial overlap in the presentation of *O. volvulus* infection in TR and END populations, the TR had more frequent acute (and possibly eosinophil-mediated) findings that may reflect differences in chronicity and immune tolerance felt to underlie the relative hyporesponsiveness seen in lifelong onchocerciasis.

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DIAGNOSIS OF LOW-INTENSITY *SCHISTOSOMA* INFECTION IN A NON-ENDEMIC SETTING USING THE ULTRASENSITIVE LATERAL FLOW TEST FOR DETECTION OF SCHISTOSOME CIRCULATING ANODIC ANTIGEN (CAA)

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Schistosomiasis in travelers and migrants is mostly diagnosed by detecting specific antibodies in serum, as microscopy has a pronounced low sensitivity in this particular population. Although serology is sensitive and specific, it cannot distinguish active from past infection and it may take up 6 to 10 weeks for seroconversion to occur. An alternative diagnostic tool is detection of adult worm-derived circulating antigen in serum or urine. Here we explored the diagnostic value of an ultrasensitive robust lateral flow based test for the quantification of the *Schistosoma* Circulating Anodic Antigen (CAA) utilizing fluorescent up-converting phosphor reporter particles (UCP-LF CAA assay) within a non-endemic routine diagnostic laboratory setting. Serum samples from 81 serology positive cases were tested, including 36 travelers of which 14 had proven seroconversion. CAA (>0.1 pg/ml) was detected in 68% of all schistosomiasis cases, including 56% of the travelers and 64% of those who seroconverted. All 19 controls were CAA negative, while all 11 subjects who were positive for microscopy and/or PCR in stool or urine were CAA positive. In travelers CAA was seen as early as four weeks after exposure and the antigen could be demonstrated in four out of five samples collected days to weeks before antibodies were observed. On the other hand, most of the CAA positive travelers (18/20) had marginally to low (<10 pg/ml) serum CAA concentrations, while higher levels were seen in the subjects with chronic schistosomiasis. Consecutive samples were tested in 16 subjects and all showed a rapid decline in CAA concentration, reflecting decreasing worm loads due to anti-schistosomal therapy. This explorative retrospective study indicates the UCP-LF CAA assay to be a highly accurate test for diagnosing schistosomiasis in a non-endemic setting.

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FIELD-TESTING OF A COST-EFFECTIVE MOBILE-PHONE BASED MICROSCOPE FOR SCREENING OF *SCHISTOSOMA HAEMATOBIUM*

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Schistosomiasis is a parasitic and neglected tropical disease, and affects >200-million people across the world, with school-aged children disproportionately affected. Here we present field-testing results of a handheld and cost effective smartphone-based microscope in rural Ghana, Africa, for point-of-care diagnosis of *Schistosoma haematobium* infection. In this mobile-phone microscope, a custom-designed 3D printed opto-mechanical attachment (~150g) is placed in contact with the smartphone camera-lens, creating an imaging-system with a half-pitch resolution of ~0.87μm. This unit includes an external lens (also taken from a mobile-phone camera), a sample tray, a z-stage to adjust the focus, two light-emitting-diodes (LEDs) and two diffusers for uniform illumination of the sample. In our field-testing, 60 urine samples, collected from children, were used, where the prevalence of the infection was 72.9%. After concentration of the sample with centrifugation, the sediment was placed on a glass-slide and *S. haematobium* eggs were first identified/quantified using conventional benchtop microscopy by an expert diagnostician, and then a second expert, blinded to these results, determined the presence/absence of eggs using our mobile-phone microscope. Compared to conventional microscopy, our mobile-phone microscope had a diagnostic sensitivity of 72.1%, specificity of 100%, positive-predictive-value of 100%, and a negative-predictive-value of 57.1%. Furthermore, our mobile-phone platform demonstrated a sensitivity of 65.7% and 100% for low-intensity infections (≤50 eggs/10 mL urine) and high-intensity infections (>50 eggs/10 mL urine), respectively. We believe that this cost-effective and field-portable mobile-phone microscope may play an important role in the diagnosis of schistosomiasis and various other global health challenges. We discuss the use of these instruments in clinical and public health settings.

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THE EMERGENCE AND EPIDEMIOLOGY OF ENDEMIC (FLEA-BORNE) TYPHUS IN TEXAS, 2003-2013

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Endemic or murine typhus, a disease spread by fleas and caused by the organism Rickettsia typhi, is a neglected tropical disease in Texas. The purpose of this study was to characterize the epidemiology of murine typhus in the state, identify seasonality of disease transmission, and identify high-risk geographic and demographic populations. We analyzed data on all confirmed and probable cases reported to the Texas Department of State Health Services' Zoonosis Control Branch between 2003 and 2013. Over this time period, 1762 cases were reported (770 confirmed and 992 probable cases). Evidence of emergence was seen both in increased numbers of cases over time (27 cases in 2003 versus 222 cases reported in 2013) and geographical location (9 counties in the southern-most part of the state in 2003 to 40 counties reporting cases by 2013). With regards to demographics, females had a slightly higher attack rate compared to males (7.3 vs. 6.7 per 100,000 population, respectively). When examining risk of disease by age, the highest attack rate (10.4/100,000 population) was found among 5-19 year olds. Of these cases, 1047 (59.6%) were hospitalized. Most commonly reported signs and symptoms included fever (99.7%), headache (77.2%), chills (70.1%), malaise (64.1%), anorexia (52.8%), nausea/vomiting (51.4%), and myalgias (50.8%). Rash was reported by 42.5% of cases, with pediatric cases being statistically more likely to present with a rash when compared to adults (odds ratio = 2.2). Fatality was rare, with 4 deaths being reported (0.2% case fatality rate). Median age of fatal cases was 51.5 years (range 36-55 years). With the increase in reported cases, high percentage of hospitalizations, and geographic expansion of transmission, we want to highlight the importance of public education and raising physician awareness to identify and treat suspected cases. Additionally, further research is needed to better understand the dynamics of transmission and risk of infection in these newly identified geographic regions.

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ZIKA VIRUS DISEASE AMONG TRAVELERS RETURNING FROM THE AMERICAS BETWEEN JANUARY 2013 AND FEBRUARY 2016: A GEOSENTINEL ANALYSIS

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Since first identified in Brazil in 2015, Zika virus (ZIKV) has rapidly spread to >30 countries and territories in the Americas. Travelers, important sentinels of new disease outbreaks, can facilitate global disease spread. ZIKV disease acquired in the Americas and evaluated at GeoSentinel Surveillance Network sites from January 1, 2013 to February 29, 2016 were analyzed. Interim Council of State and Territorial Epidemiologists classification criteria were used to classify patients as clinically suspect, probable, or confirmed cases of ZIKV disease. There were 62 confirmed, 13 probable, and 16 clinically suspect ZIKV cases of 100 cases submitted. Of the 91 cases, 64% were female, and median age was 41 y (range 3-77 y). Major reasons for travel were tourism (48%) and visiting friends and relatives (40%). Infections were acquired in South America (59%), the Caribbean (24%), and Central America/Mexico (16%); the top three countries were Suriname (22%), Colombia (16%), and Brazil (11%). Diagnoses were made in Western Europe (73%), North America (15%), Middle East (9%), and South America (3%). The first two cases were reported in May 2015 and November 2015, after which there was a rapid rise in cases. The most common sign/symptoms were rash (88%), fever (76%), arthralgia (72%), headache (60%), myalgia (60%), fatigue (47%), conjunctivitis (41%), and pruritus (23%). Less common were nausea, diarrhea, paresthesia, dysgeusia, and arthritis. Of 4 pregnant patients, 2 had normal ultrasounds, one underwent elective termination due to major fetal neurological abnormalities, and one had no documented outcome. Two patients were diagnosed with Guillain-Barré syndrome. This large case series demonstrates the range of symptoms associated with ZIKV infection. While sentinel surveillance does not reflect true incidence and is biased towards identifying more severe disease and influenced by testing availability, GeoSentinel data are important for tracking the geographic spread of emerging infections. We show that travelers infected in the Americas return to locations where they can potentially transmit the virus to sexual partners or competent vectors.

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OUTCOMES OF PREGNANT PATIENTS PRESENTING TO EBOLA TREATMENT UNITS IN SIERRA LEONE AND LIBERIA: A RETROSPECTIVE COHORT STUDY

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Pregnant women are thought to have increased risk for severe illness and death when infected with Ebola virus disease (EVD). This retrospective cohort study investigated the presenting symptoms and outcomes of pregnant women compared with non-pregnant women of reproductive age admitted to five Ebola Treatment Units (ETUs) in Sierra Leone and Liberia between September 2014 - 2015. Data analysis was completed for women of reproductive age with results for EVD testing, with a focus on those with the report of pregnancy. Standardized care, including empiric malaria treatment and oral antibiotics, was provided to all patients based on International Medical Corps protocols. Among 2,323 patients admitted with outcome data, 723 women of reproductive age were included for analysis. Forty-four women were documented as pregnant; the median gestational age reported was 26 weeks (range of 4 to 40 weeks). There was no significant difference in overall mortality between pregnant and non-pregnant women (13.9% vs 18.9%, p=0.42). Pregnant patients were no more likely to have EVD than non-pregnant women (29.6% vs 23.9%, p=0.39), and were less likely to have fever, bone/muscle pain, nausea, vomiting, diarrhea, anorexia and asthenia as presenting symptoms when compared to non-pregnant women. Thirteen pregnant patients were EVD positive. Six died, six survived to discharge, and one was transferred to a nearby ETU with specialized care for pregnant women (outcome unknown). There was no difference in mortality between pregnant and non-pregnant women with EVD (50% vs 53.7%, p=0.80). There were two live births in the ETU; both infants died before hospital day 9. Training guidelines for providers working in Ebola treatment units focus on maternal deliveries without assistance to minimize risk to healthcare workers considering historical data suggested poor maternal or fetal survival. Our data points to comparable maternal outcomes, though fetal survival in the context of an EVD gestation remained poor. Based on this data, re-evaluating the approach to management of pregnant patients in the ETU setting may be warranted.

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DECIPHERING THE BIOLOGY OF THE DORMANT MALARIA PARASITE, *PLASMODIUM VIVAX*, VIA AN *IN VITRO* PLATFORM

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Liver stage malaria is an attractive target for all *Plasmodium* species since it provides the opportunity to attack the parasite at an early, obligate, yet clinically silent stage. Of the 4 strains of malaria that infects humans, *P. vivax* is the most frequent and widespread cause of human malaria and poses unique challenges to treatment and eradication because liver stage parasites can develop into dormant small forms, hypnozoites, that remain in this state for prolonged periods. This strain of malaria causes chronic, relapsing infection months to years after the initial infection via reactivation of hypnozoites. Drug discovery could be informed by targeting the hypnozoite stage, however the biology behind hypnozoite formation and reactivation remains to be elucidated. *In vitro* platforms

for studying *P. vivax* are lacking because of challenges associated with keeping primary human hepatocytes phenotypically stable over the long periods of time required for studying dormant parasites. Benefiting from the longevity of our micropatterned primary human hepatocyte cultures, we were, for the first time, able to culture *P. vivax* hypnozoites *in vitro*. To validate the system as an *in vitro* surrogate for *P. vivax* biology applications, we have shown (1) complete *P. vivax* liver stage development, including release of merozoites and subsequent infection of overlaid reticulocytes and (2) formation, persistence and reactivation of hypnozoites. Using the system as a potential discovery tool, we presented evidence of differential drug sensitivity of schizonts and hypnozoites towards both clinically available and yet-in-development liver-acting drugs. Furthermore, leveraging the power of an *in vitro* culture in facilitating rapid testing of biological hypotheses that are otherwise clinically difficult to test, we created a simple deterministic model that recapitulates the behavior of *P. vivax* parasites over time. Fitting experimental data into this model, we addressed two hallmarks of *P. vivax* liver stage biology: lifetime in the liver and hypnozoite reactivation frequency.

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CELL TRAVERSAL BY MALARIA PARASITES: *PLASMODIUM* CELTOS BINDS AND DISRUPTS PLASMA MEMBRANES FROM THE CYTOPLASMIC FACE TO ENABLE THE EXIT OF PARASITES FROM CELLS DURING HOST AND VECTOR CELL TRAVERSAL

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Traversal of host and vector cells by *Plasmodium* parasites is required for malaria infection and transmission. *Plasmodium* CelTOS has been identified by genetic studies to be critical for the traversal of parasites through the mammalian host and mosquito vector. Additionally, CelTOS is a leading malaria vaccine candidate in clinical trials. Even though CelTOS has critical roles in *Plasmodium* biology and is a malaria vaccine target, the molecular function and mechanism of CelTOS remains unknown primarily due to its lack of sequence similarity to proteins of known function. We determined the structure of *Plasmodium* CelTOS to obtain insight into its molecular function. Unexpectedly we discovered CelTOS is structurally similar to viral fusion proteins and a bacterial pore-forming toxin that bind membranes. Unlike other membrane binding proteins, CelTOS specifically lipids predominantly present in the inner leaflet of plasma membranes. We also observed that CelTOS disrupts liposomes composed of these lipids, and further *in vivo* studies demonstrate that CelTOS disrupts cell plasma membranes. Taken together, these studies demonstrate that CelTOS is the only known malaria parasite protein that enables the exit of parasites from host and vector cells during traversal by having nearly universal activity in binding and disrupting plasma membranes from the cytoplasmic face. By providing insight into the function and mechanism of CelTOS, this study facilitates the design of therapeutics which target CelTOS to protect against malaria.

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MECHANISM OF FETAL GROWTH RESTRICTION IN PLACENTA MALARIA

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Placental malaria can trigger intervillositis; a local inflammatory response more strongly associated with fetal growth restriction (FGR) than placental malaria infection alone. The underlying mechanisms are unknown but we have shown that placenta malaria-associated intervillositis impairs placental amino acid transport. The mechanistic target of rapamycin (mTOR) signaling pathway regulates fetal growth by modulating transplacental amino acid transport. mTOR activity has also been observed to be decreased in non-malarial cases of FGR. We hypothesized that placenta malaria-associated intervillositis inhibits mTOR signaling pathway, resulting in reduced placental amino acid uptake. Using placental tissue biopsies from Malawian women, we demonstrated that mTOR signalling activity is i) decreased specifically in placenta malaria-associated intervillositis compared to uninfected controls ($p \leq 0.03$); ii) negatively correlated with the degree of inflammation ($p \leq 0.03$; $R < -0.42$) and iii) positively correlated with amino acid uptake ($p \leq 0.03$; $R > 0.36$). Using our established *in vitro* model of placental response to intervillositis, we demonstrated that primary human trophoblast (PHT) cells exposed to placental malaria-associated intervillositis have decreased mTOR signalling activity ($p \leq 0.02$) and reduced amino acid uptake (-63%, $p \leq 0.02$), recapitulating our *ex vivo* findings. Furthermore, constitutive mTOR activation (by silencing the endogenous inhibitor of mTOR) partially restores amino acid uptake (+30%, $p \leq 0.0001$) in PHT cells exposed to placental malaria-associated intervillositis. In summary, we determined that inhibition of placental mTOR activity mechanistically links placental malaria-associated intervillositis and reduced amino acid transport, which may contribute to the pathogenesis of FGR. We propose that restoring mTOR signaling in placental malaria may increase fetal growth and complement malaria control strategies to improve pregnancy outcomes in pregnant women exposed to malaria.

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THROMBOSPONDIN RELATED SPOROZOITE PROTEIN IS IMPORTANT FOR THE ESTABLISHMENT OF *PLASMODIUM FALCIPARUM* LIVER STAGE INFECTION

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From oocyst to vertebrate host cell, malaria sporozoites must traverse and recognize multiple cell types if they are to successfully continue the parasite's lifecycle. Proteins previously recognized to be important for sporozoite-host-cell interactions; circumsporozoite protein (CSP) and thrombospondin related anonymous protein (TRAP), contain thrombospondin type 1 repeats (TSR). Another less well characterized TSR protein, highly expressed in sporozoites, is thrombospondin related sporozoite protein, which contains a TSR, a transmembrane domain and a putative PEXEL motif, suggesting it may localize to the surface of the sporozoite and/or be exported. TRSP has previously been shown to be important for the successful invasion and establishment of liver stage infection in *P. berghei*. In order to investigate the role and localization of this protein in *P. falciparum*, the species responsible for the vast majority of human malarial morbidity and mortality worldwide, we generated

P. falciparum TRSP knockout parasites, as well as lines expressing GFP and HA tagged TRSP. TRSP deficient parasites show no defect until the sporozoite stage, where they display a mild hepatocyte traversal defect *in vitro*. This does not however result in an *in vitro* invasion defect as seen in *P. berghei*. To further investigate the importance of this traversal defect *in vivo*, ΔTRSP sporozoites were injected into uPA/SCID mice with humanised livers, where a 95% reduction in parasite load was observed six days post infection by qPCR. Preliminary data indicate that ΔTRSP liver schizonts can develop with a morphology and size similar to wildtype and morphological characteristics will be discussed. These data show that TRSP, in common with other TSR containing sporozoite proteins, is important for the establishment of liver stage infection, most likely during the invasion process, however work is ongoing to further define the exact function of TRSP in this important human pathogen.

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A SYSTEMATIC APPROACH TO THE OPTIMIZATION OF *PLASMODIUM VIVAX IN VITRO* CULTURE

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The WHO estimates that 35% of the global population was at risk for and approximately 16 million people suffered from clinical *Plasmodium vivax* infection in 2013, making *P. vivax* the most globally widespread malaria parasite and an immense health and economic burden. Unfortunately, largely due to its unique biology, a continuous, *in vitro* culture system for this parasite has remained elusive, hindering our ability to make scientific advancements and combat *P. vivax*. A major obstacle to continuous, *in vitro* culture is our lack of understanding the nutrient requirements of *P. vivax* for successful asexual maturation and survival. To enhance *P. vivax ex vivo* growth and to understand the nutrient requirements of the parasite, we designed and executed a screen to identify an optimal culture media. Twenty different media were commercially acquired that represent a range of defined and proprietary formulations, with an emphasis on media developed for cultivation of erythrocytes and erythrocyte precursors. From this screen, we identified a formulation that significantly enhances the survival and asexual maturation of human *ex vivo P. vivax* isolates from ring stages to schizogony and egress. Furthermore, the increased survival and maturation has enhanced our ability to perform invasion assays. While it has been previously demonstrated that *P. vivax* preferentially invades very young red blood cells, reticulocytes, our assays indicate that a currently uncharacterized, more specific subset of reticulocytes is required for efficient invasion and subsequent asexual maturation. To enable the identification of a host cell that fully enables *P. vivax* invasion and maturation, we have developed methods to fractionate very specific subpopulations of human reticulocytes from multiple sources representing the full range of erythropoiesis. In all, this systematic approach to the optimization of *in vitro* culture of *P. vivax* has enabled us to reliably perform short-term growth assays, establish more robust invasion assays, and it has brought us closer to continuous *in vitro* culture.

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MOLECULAR TECHNIQUES IDENTIFY *ANCYLOSTOMA CEYLANICUM* AND *NECATOR AMERICANUS* AS THE MAJOR HOOKWORM PATHOGENS AMONG MYANMAR REFUGEES PRE-RESETTLEMENT AND DEFINE THEIR DIFFERENTIAL RESPONSE TO ANTHELMINTIC THERAPY

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Human hookworm infection, typically caused by *Necator americanus* (Na) and *Ancylsotoma duodenale* (Ad), remains an important source of childhood growth restriction, iron deficiency, and anemia. Using multi-parallel real-time PCR (qPCR) on stool samples from 233 subjects that were part of a larger study (n=2000) assessing the prevalence of intestinal parasites in refugees from Myanmar (domiciled in refugee camps in Thailand) and the efficacy of pre-resettlement anthelmintic therapy, we assessed Na and Ad prevalence in all subjects from 3 time points (1-following arrival in refugee camps in Thailand, 2-after treatment with albendzaole/ivermectin (A/I) prior to departure for the United States (US), and 3-following arrival in the US after a second treatment with A/I). At entry, 51 of 233 (22%) were infected with Na. QPCR targeting the ITS2 region of *Ancylostoma* spp. revealed 17 of 233 (7%) stool samples were positive upon entry to the refugee camp. However, qPCR targeting a repeated sequence specific for *A, duodenale* was negative for all 17 samples. Sequencing and follow up qPCR revealed all 17 of these samples to be positive for the zoonotic hookworm *A. ceylanicum* (Ac). Following A/I therapy, all subjects with Ac cleared their infection. Of the 51 subjects infected with Na at baseline, 20 (39%) remained infected with Na following the first A/I dose and 14 (27%) remained Na-infected despite 2 courses of A/I. Six previously hookworm-uninfected subjects acquired Na infection while in the refugee camp (positive at timepoint 3), and 2 acquired Ac (positive timepoint 2). Studies are ongoing to sequence the beta-tubulin genes of those Na parasites that responded and failed to respond to A/I. These data identify the zoonotic Ac as an emerging and important human pathogen in Myanmar. Thus, the availability of increasingly sophisticated molecular diagnostic techniques to assess the differential responses of the various hookworm species to anthelmintics and to provide evidence of ongoing acquisition of hookworm infection within refugee camps should guide future planning of pre-departure medical treatment for refugees in Southeast Asia.

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MULTI-PARALLEL QUANTITATIVE REAL-TIME PCR BASED DIAGNOSTICS AS THE NEW GOLD STANDARD FOR SOIL TRANSMITTED HELMINTHS

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Due to its simplicity and cost-effectiveness, microscopy has seen extensive field-use as the diagnostic standard for the detection of soil-transmitted helminths (STH) in stool samples. However, the sensitivity of microscopy-

based detection is inadequate in reduced-transmission settings where worm burden is oftentimes low. Equally problematic, eggs of closely related species oftentimes have indistinguishable morphologies, leading to species misidentification. In light of these shortcomings, the purpose of this study was to demonstrate multi-parallel quantitative real-time PCR (qPCR) as the new "gold standard" for STH detection. Accordingly, stool samples from non-endemic participants were spiked with limited numbers of eggs or larvae (1 to 40) of five different species of STH. DNA extracts were tested using two unique multi-parallel real-time PCR-based diagnostic methods. These methods employed different target sequences (ribosomal internal transcribed spacer, or highly repetitive non-coding regions), to evaluate the detection of DNA from as little as one egg per sample. There was a statistically significant spearman correlation between egg/larvae counts and qPCR from both methods for each one of the multi-parallel assays; for Ascaris lumbricoides (0.806), *Ancylostoma duodenale* (0.961), *Necator americanus* (1.00), *Strongyloides stercoralis* (0.835), and *Trichuris trichiura* (0.956) ($p < 0.05$ for all STH). Both methods had similar detection rates for 104 stool samples from a rural population in northern Argentina with less than a 25% variance between them for most STH. As parasitic targeting of two independent genomic regions provided reproducible results, we believe that, low cost multi-parallel quantitative real-time PCR-based diagnostics should supplant microscopy as the new gold standard for stool-based detection of soil transmitted helminths in public-health and community settings.

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DIAGNOSIS OF *STRONGYLOIDES STERCORALIS* FROM FILTERED URINE RESIDUE BY DETECTING CELL-FREE DNA

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One of the most neglected of the neglected tropical diseases is the infection caused by nematodes in the genus *Strongyloides*. Of the two species of *Strongyloides*, *S. stercoralis* is the most prevalent that infect humans and found in tropical and subtropical regions. Detection of *S. stercoralis* infection is arduous and has low sensitivity. This is a major problem because chronic infections may disseminate in the host and lead to a life threatening condition. Even now, the true prevalence of this parasite is practically unknown because the standard practice for stool examinations usually misses evidence of this infection. We present here the evidence for the first time that the infection can be detected by amplifying *S. stercoralis* specific cell-free repeat DNA from urine residue on filter paper that has been collected and processed in the field. We collected 125 specimens from people living in two types of endemic regions in Northern Argentina (rural and peri-urban). Stool specimens were processed fresh using three different coprological methods and 40-50 ml of urine was filtered through a 12.5cm Whatman No. 3 filter paper in the field. The filters were dried and packed individually in sealable plastic bags with desiccant and shipped to Johns Hopkins University where DNA was isolated and amplified with species-specific primers. The estimate of prevalence of infection was almost doubled when detecting the *S. stercoralis* specific repeat compared with coprological diagnosis (from 28% to 44.8%). Only 21.6% positive cases were congruent, as were 27.3% of negative cases. There were 6.4% of cases where parasite larvae were seen but DNA was not amplified. The species-specific DNA detection from urine residue reveals significantly more cases of infection than combined stool examinations and the method is simple and easy to carry out. This is crucial not only to determine the overall public health impact of the pathogen, but also to understand the extent of the infection in communities so that attempts to control or eliminate these pathogens are efficient and cost-effective.

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MATERNAL POSTPARTUM DEWORMING AS A MEANS OF IMPROVING INFANT GROWTH AND MORBIDITY IN AREAS ENDEMIC FOR SOIL-TRANSMITTED HELMINTHIASIS

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Health and nutrition interventions targeting the critical growth and development period before the age of two years can have the greatest impact on health trajectories over the life course. Compelling evidence suggests that interventions in the postpartum period can be beneficial for both mothers and their children. One such potential intervention is deworming. In this seminal randomized controlled trial, we sought to evaluate the effectiveness of maternal postpartum deworming on infant growth. A total of 1010 mother-infant pairs were recruited in Iquitos, Peru. Prior to delivery, mothers provided stool specimens to determine the presence of soil-transmitted helminth (STH) infection. Following delivery, mothers were randomly assigned to receive either single-dose 400 mg albendazole, or matching placebo. Mother-infants pairs were followed-up at 1 and 6 months postpartum. At 6 months postpartum, there was no statistically significant difference in mean weight gain between infants in the albendazole and placebo groups (4.3 kg ± 0.04 *vs.* 4.4 kg ±0.04). However, *ad hoc* subgroup analyses restricted to mothers who tested positive for STH infections at baseline suggest that infants whose mothers received albendazole had greater growth in terms of mean length gain in cm (mean difference: 0.8; 95% CI: 0.1, 1.4) and length-for-age in Z-score (mean difference: 0.5; 95% CI: 0.1, 0.8). In a study population composed of both infected and uninfected mothers, maternal postpartum deworming with single-dose albendazole was insufficient to impact infant growth indicators at 1 or 6 months of age. Among STH-infected mothers, however, important infant growth benefits were observed. The benefits of postpartum deworming should be further investigated in study populations having different prevalences and intensities of STH infections and, in particular, where the prevalences and intensities of whipworm and hookworm infections are of public health concern.

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SOIL-TRANSMITTED HELMINTHS (STH) CONTROL, ELIMINATION, AND DEVELOPMENT OF DRUG RESISTANCE: REPERCUSSIONS OF SYSTEMATIC NON-PARTICIPATION TO PREVENTIVE CHEMOTHERAPY

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Preventive chemotherapy (PCT) is a widely applied strategy to control soil-transmitted helminths (STH). However, mathematical models predict that effective control and/or elimination are easily impeded by suboptimal PCT coverage and/or systematic non-participation to PCT by a subset of individuals. Further, suboptimal coverage and systematic

non-participation may on the one hand facilitate the development of drug resistance through prolonged programme duration until elimination, but on the other hand may also delay it by allowing for parasite refugia in suboptimally treated populations. We present a novel individual-based model for evolution of polygenic drug resistance in helminth populations that accounts for aggregation of helminths in human hosts, human demography, patterns in PCT uptake (including systematic non-participation), sexual mating of parasites, genetic drift, and variation and heritability of parasite traits for drug resistance. The model has been quantified for transmission and control of the three major STH species (ascariasis, trichuriasis, hookworm), and has been used to simulate and explore the impact of various levels of drug efficacy, PCT coverage, and systematic non-participation to PCT on the speed at which polygenic drug resistance evolves in STH populations under varying assumptions about variation and heritability of parasite traits for drug resistance. Based on these explorative simulations, we provide a first-time estimate of the time horizon within which we can expect polygenic drug resistance to develop in STH populations as a result of PCT.

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DIFFERENTIAL IMPACT OF MASS AND TARGETED DEWORMING CAMPAIGNS FOR SOIL-TRANSMITTED HELMINTH CONTROL IN CHILDREN: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Soil-transmitted helminth (STH) infections are an important global health issue, causing significant morbidity among the world's poorest populations. Regular delivery of anthelminthic chemotherapy is the principal strategy for STH control. As children harbour the largest burden of STH-associated morbidity, deworming medications are often targeted to school-aged children. However, recent modelling studies suggest that deworming campaigns should be expanded community-wide to have an impact on STH transmission.This systematic review and meta-analysis aimed to compare the impact of community-wide and child-targeted anthelminthic delivery strategies on STH prevalence in school-aged children.Studies reporting STH prevalence before and after child-targeted or community-wide treatment were identified searching MEDLINE, EMBASE, and Web of Science. Data extracted included drug administration strategy, drug dose, number of deworming rounds, treatment coverage, diagnostic method, follow-up interval, and STH prevalence before and after treatment. Inverse variance weighted generalised linear models were used to examine the impact of community-wide vs child-targeted drug administration on prevalence reduction in school-aged children.56 studies were included. Results of the regression models show a significantly greater prevalence reduction in children following community-wide deworming, compared to child-targeted deworming, for both Ascaris lumbricoides (OR 16.39; 95%CI 2.14-125.85) and hookworm (OR 4.62; 95%CI 1.85-11.57).The results of this meta-analysis suggest that expanding periodic chemotherapy for STH from child-targeted to community-wide is likely to result in reduced prevalence of STH among the high-risk group of school-aged children, which may lead to decreased morbidity. Findings are in support of recent calls for a re-evaluation of global STH control guidelines.

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RESULTS OF A COMBINED PUBLIC HEALTH INTERVENTION AGAINST *STRONGYLOIDES STERCORALIS* IN AN ARGENTINIAN ENDEMIC REGION MONITORED THROUGH NIE-ELISA

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Northwestern Argentina is a hyperendemic area for soil transmitted helminth infection, where annual deworming programs are carried out in prioritized areas for its control. In the particular case of *Strongyloides stercoralis*, high prevalence was reported in this area (30-50%); therefore, ivermectin has been included in the chemotherapy. In this context, assessing the *S. stercoralis* response to therapy in the treated population has become a major concern. The NIE ELISA was used for this purpose since it proved to be more sensitive than conventional parasitological techniques for the diagnosis of *S. stercoralis*. This community trial was conducted in two groups of patients, classified according to differences in housing and living conditions. After the first sampling and deworming (Massive Drug Administration, MDA), Group 1 (G1) was moved to new households with drinking water access and improved sanitation facilities (MDA+WAS intervention); while Group 2 (G2) remained living in less developed conditions with unimproved drinking water and sanitation (MDA intervention). The mean interval times between baseline (Base) and the follow up (F/U) were 359 days for G1 and 478 for G2. Anti-NIE antibody titers (Optical Density, OD) were measured for each individual before and after interventions (paired sera). A follow up OD ratio ($OD_{F/U}$/OD_{Base}) was calculated to quantify the variation in antibody titers. A significant decrease of the anti-NIE titers ($p<0.0001$) between Base and F/U was observed in both groups. Nonetheless the number of patients who achieved the cure criteria (ODratio<0.6) was different between groups: G1: 75% (24/32); G2: 45% (17/38) ($p=0.038$). We found that NIE ELISA is a useful test for assessing the response to treatment and to evaluate the outcome of control interventions in the population. Following the anthelminthic treatment, we could observe a marked decrease of anti-NIE antibodies over the time. Furthermore, our results support that a combined intervention including deworming and improvements in life conditions is more effective, in terms of number of subjects cured, than deworming only.

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COMPARISON OF ALPHAVIRUS AND FLAVIVIRUS PREVALENCE IN WESTERN KENYA

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Chikungunya virus (CHIKV) and dengue virus (DENV) are emerging mosquito-borne viruses that are endemic in tropical environments, such as Africa, Asia, South America, and the Caribbean. In rural areas of Africa, DENV and CHIKV infections often go undiagnosed and unreported, as fever presentation is commonly assumed to be a sign of malaria. The goal of this study was to measure and compare the seroprevalence of CHIKV and DENV (serotypes 1-4) among children (ages 5 to 14, n=250) and adults (ages 15 to 85, n=250) in a rural village community centered around Busia, Kenya. Samples were screened for anti-CHIKV and anti-DENV IgG by indirect ELISA. As expected, children were less likely to be

exposed to CHIKV ($p < 0.001$) than adults. For children, 141 samples (56.4%, CI95 0.500 to 0.626) were positive for anti-CHIKV IgG, and 2 samples (0.8%, CI95 0.001 to 0.029) were positive for anti-DENV IgG. Comparatively, 195 samples (78.0%, CI95 0.724 to 0.83), and 6 samples (2.4%, CI95 0.009 to 0.052) of the 250 samples from adult participants were positive for anti-CHIKV IgG and anti-DENV IgG, respectively. Overall, 67.0% of participants showed seropositivity for CHIKV (CI95 0.627 to 0.711), and 1.6% of participants were seropositive for DENV (CI95 0.007 to 0.031). These results confirm the presence of alphavirus and flavivirus exposure in western Kenya, and illustrate a significantly higher severity of transmission compared to previous studies. Given the expansive spread of the endemic in recent years, understanding the true severity, prevalence and burden of infection of DENV and CHIKV is critical for predicting the future impacts of each virus.

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SEROPREVALENCE OF FLAVIVIRUSES AND ALPHAVIRUSES IN CHILDREN IN COASTAL KENYA: A 2015 SNAPSHOT

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Some of the most emergent and destructive diseases are mosquito-borne viruses. The non-specific symptoms of such viral infections lead to misdiagnosis and minimal case reporting, making the true impact of such infections difficult to determine. This cross-sectional study aims to describe the true prevalence of flaviviruses, such as dengue virus (DENV) and West Nile virus (WNV), and alphaviruses, such as chikungunya virus (CHIKV) and o'nyong n'yong virus (ONNV), in an urban community on the coast of Kenya in 2015. A subset of 700 afebrile children, aged 1-17 years, was selected from an ongoing community cohort study. Questionnaire data, including health history, socioeconomic status, home environment, and mosquito exposure, was used to determine potential risk factors associated with exposure to alpha- and/or flaviviruses. InBios CHIKjj Detect™ and DENV Detect™ IgG ELISA kits were used to identify IgG antibodies against DENV1-4 and CHIKV in follow-up samples. IgG seroprevalence was 2% for CHIKV (CI95 0.8-2.9%) and 1.4% for DENV (CI95 0.6-2.6%). Genus-specific cross-reactivity is anticipated with IgG ELISAs. Seropositivity for anti-CHIKV IgG, indicating previous alphavirus exposure, was associated with frequent outdoor activity ($p=0.003$) and lack of utilization of mosquito avoidance measures ($p=0.025$). Seropositivity for CHIKV ($p=0.025$) and DENV ($p=0.046$) was associated with mosquito bites at night. Gender was not significantly associated with prior alpha- or flavivirus exposure. Children as young as 5 were seropositive for either anti-CHIKV or anti-DENV IgG, indicating active alpha- and flavivirus transmission within the last 5 years. Children aged between 7 and 12 years were more likely to be seropositive for anti-CHIKV and anti-DENV IgG ($p<0.001$) when compared to younger participants. Prevalence data may not accurately represent the severity of exposure, infection, and disease throughout Kenya, as the varied reports of prevalence in other regions indicates differences that may be dependent on geographic region. These results confirm the continued presence of alphavirus and flavivirus exposure in children in coastal Kenya.

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CHIKUNGUNYA VIRUS INFECTION IS CAUSING ACUTE FEBRILE ILLNESS AMONG CHILDREN IN KENYA

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Several chikungunya virus (CHIKV) outbreaks have occurred in Africa, Asia, and the Americas in the last decades. However, due to limited surveillance, few serologic data are available in Africa and as a result, there is a gap in our understanding of ongoing endemic transmission of CHIKV. Thus the risk for CHIKV infection in African peoples remains unknown. In order to prevent disease emergence, the dynamics of CHIKV infection must be understood and the risk factors for transmission need to be evaluated. For our study, we enrolled children with acute febrile illness who presented to one of four Kenyan health centers (in Chulaimbo and Obama Children's Hospital in western Kenya, and Msambweni and Ukunda on the Kenyan coast). In each region, one of the sites is localized in an urban area and the other in a rural area, in order to determine whether the differences between the two environments have an effect on the transmission rate of CHIKV infection in humans. Serum samples were collected at an initial visit and at a one-month follow-up visit for CHIKV ELISA testing. Questionnaire data were collected to describe demography, education, and household environment, along with clinical data. In our preliminary screening of 125 paired acute and convalescent serum samples by ELISA for anti-CHIKV IgG, we identified 5 cases (4%, 95% CI 1.3% to 9.1%) of seroconversion. These cases demonstrate recent active transmission of CHIKV in Kenya, both on the coast and in the west. Because of the small number of seroconversions in our preliminary analyses, we did not identify any differences in risk of infection associated with either urban or rural locale. We also did not detect any link between exposure to different water sources and seroconversion, however we did find that people who use a river or a pond as water source were more likely to report mosquito bites than people who have access to a public well (88.7% vs 70.2%, respectively, $p<0.0001$ by Fisher's test). Further testing may reveal important risk factors for seroconversion and will help identify potential interventions to reduce risk of developing CHIKV infection in Kenya.

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IDENTIFICATION OF FACTORS ASSOCIATED WITH CHRONIC CHIKUNGUNYA DISEASE IN PATIENTS IN GRENADA, WEST INDIES

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Chikungunya virus (CHIKV) is a rapidly re-emerging arboviral pathogen worldwide. In July 2014, an explosive CHIKV outbreak occurred in Grenada, West Indies, infecting around 90% of the population, with a wide spectrum of disease reported. In an estimated 50% of cases, CHIKV infection transitions to a non-communicable painful arthritis that can persist for years. Our understanding of the risk factors and mechanisms underlying chronic disease are limited. Here, we conducted one-year follow up with 240 people who were tested for CHIKV during the Grenada outbreak and performed analyses on demographic, behavioral, exposure, and co-morbid health factors to identify associations with chronic disease. Physical examinations were performed and current arthritis/arthralgia symptoms, as well as prior medical history was recorded. Participants

also completed extensive questionnaires so that physical, psychological, social and environmental factors could also be assessed. "Chronic" CHIKV disease cases were defined as individuals who continue to experience arthralgia and/or arthritis >6 months after onset of their acute CHIKV disease that impacts activities of daily living. Demographic factors including age (p=0.56), gender (0.058), ethnicity (0.58) and socioeconomic status did not have an effect on the likelihood of suffering from chronic persistent CHIKV disease. Increased mosquito avoidance behavior also did not reduce the risk of chronic sequelae. Patients who suffered joint pains (0.005), muscle pains (0.042), generalized body ache (0.013) and weakness in the extremities (0.013) during acute CHIKV disease were more likely to have chronic arthritis and arthralgia symptoms, and an increased duration of acute disease (0.001) also increased risk. None of the co-morbidities measured were associated with increased disease risk. These data demonstrate that chronic CHIKV disease affects people across the age, gender, ethnic and socioeconomic spectrum, and is not reduced by vector avoidance activity. Management of acute symptoms and minimization of acute disease duration could reduce chronic sequelae.

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SIMULATING CHIKUNGUNYA OUTBREAKS IN COLOMBIA USING AN AGENT-BASED MODEL

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The first chikugunya epidemic in the Americas was reported between 2013 and 2016. In this period, around 1.7 million clinical cases were reported. Due to the lack of a vaccine, vector control was the only resource to halt the epidemic. The response of governments to the emergency faced challenges because of uncertainty in the possible magnitude of the epidemic and in the cost-benefit ratio of vector control programs. We developed an agent-based model as a tool to predict transmission dynamics of vector-borne diseases and to assess the value of vector control. We applied the model to chikungunya in Colombia, where half a million cases were reported. By representing large-scale populations with an agent-based model, we are able to reproduce population-level patterns of the epidemic while accounting for heterogeneity of transmission using high-resolution demographic, geographic, and climate characteristics of the population. Also, our model allows for the evaluation of the impact on the epidemic of individual actions, such as vector control. In the model, transmission of the virus occurs upon human contacts with mosquitoes in specific locations. We used population density and climate grids to reproduce the mosquito abundance in space. Moreover, we created and calibrated a synthetic population to represent human demographics, contact patterns, and activities of 45 million inhabitants of Colombia. We calibrated the model with and without vector control to incidence reports of the first 24 weeks of the outbreak. To predict the possible spread of the epidemic, we evaluated various schemes of geographical distribution of vector control. Also, we validated the model predictions using the incidence reports in Colombia available from 2014 to early 2016. These simulation results show that giving priority to dengue-endemic areas, notably reduces the impact of the epidemic. Furthermore, the model predicted the shape and magnitude of the incidence curves reported in five out of six regions of Colombia. Finally, we believe that this platform can be used to evaluate the impact of similar transmitted viruses such as zika or dengue.

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VACCINES AGAINST EMERGING ALPHAVIRUSES

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Emerging and re-emerging alphaviruses are responsible for several important infectious diseases. Although previously confined to mainly tropical areas, the geographic occurrence of alphaviruses now overlaps temperate regions including urban areas due to environmental and ecological changes, vector-host interactions and more competent viral strains. While some alphavirus infections can be asymptomatic, others such as those caused by Chikungunya, Ross River or O'nyong'nyong virus can cause high fevers, debilitating myalgia, rashes and arthritic disease. In addition, these infections are also accompanied by more severe symptoms such as life-threatening encephalitis, myocarditis or hemorrhagic fevers. At present, there is no specific FDA-approved medical treatment for infection with these viruses although some concerted efforts are directed at vaccine development. AC Immune has developed a number of various vaccines that can generate robust and long lasting antibody responses, independently of T cells. T cell responses raised during natural infection or alphavirus vaccination have been linked to more severe pathology. Moreover, T cell mediated responses are likely associated with brain encephalitis and arthritic disease. Therefore, a highly desirable feature in alphavirus vaccines is to induce a robust antibody response that can neutralize the virus infection and confer lifelong protection against recurrent re-infections, while avoiding reactive T cells. We have designed new vaccines with different linear and conformational relevant CHIKV peptide sequences. Results in murine models suggest that robust and long-lasting antibody responses are raised upon vaccination and are accompanied by low or no T cell responses. Therefore, our vaccines show promising results against alphaviruses causing arthritis-like symptoms, as well as other infectious diseases where T cell mediated responses are preferably avoided.

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IMMUNE PROFILING AND NETWORK MODELING OF CHIKUNGUNYA INFECTION IN A HOSPITAL-BASED STUDY IN NICARAGUA

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Chikungunya virus (CHIKV) is an important emerging, mosquito-borne arithrogenic alphavirus that causes both explosive epidemics of debilitating rheumatic disease with fever, asthenia, skin rash and occasionally more severe complications that can be fatal. In December 2013, an outbreak of chikungunya in the Caribbean was reported; since then, over one million CHIKV cases are estimated to have occurred, and most countries of the Americas are reporting autochthonous transmission of CHIKV. It is of global concern that the chikungunya epidemic in the Americas is still

growing and the number of CHIKV-infected people is rising every year, yet much remains to be defined regarding the human immune response to CHIKV infection, especially in children. In our study, we collected acute (day 2 or 3) and convalescent (day 15 or 16) samples from symptomatic CHIKV-infected pediatric cases (n=42) presenting to the national pediatric reference hospital in Managua, Nicaragua. Comprehensive innate and adaptive immune responses were investigated by CyTOF, Luminex cytokine assays, and RNA-seq. Initial analyses revealed that the frequencies and phenotype of several major circulating leukocytes, particularly monocytes and dendritic cells, as well as the cytokine and chemokine profile, are significantly different between acute and convalescent samples. Additionally, we have performed RNA-seq analysis at both phases of CHIKV infection to identify differences in gene expression at the transcriptomic level. These data are being analyzed to identify immune signatures and potential biomarkers of CHIKV infection. All data are being integrated for network modeling, consisting of weighted gene co-expression network analysis (WGCENA), dynamic Bayesian networks and key driver analysis to generate global, unbiased maps of regulatory relationships and to uncover novel host-virus pathways and driver genes. Our study will provide the most comprehensive immune profiling and network analysis of the human response to CHIKV infection to date and will help inform future diagnostics and drug therapies.

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ATYPICAL CHIKUNGUNYA PRESENTATION DURING THE 2014 EPIDEMIC IN VENEZUELA

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In 2014, Venezuela witnessed one of the worst epidemics ever seen caused by a mosquito-borne virus: Chikungunya (CHIK). The virus rapidly spread through the country affecting ~60% of the population. Although CHIK was not considered a severe disease, during the epidemic in Carabobo State, one of the first Venezuelan regions to be affected, patients developing atypical CHIK presentation were soon observed. We aimed to characterize the atypical clinical presentation of these patients and their possible association with CHIK virus infection. Data on socio-epidemiological factors, clinical presentation, co-morbidities, laboratory parameters and other paraclinical investigations were collected after informed consent from patients admitted to the main tertiary hospital of Valencia, the capital of Carabobo State, between September-December 2014. We present data on 16 patients with a mean age of 59 years (range: 34-81) and 62.5% males. All patients reported symptoms compatible with a previous or current CHIK infection. All presented fever and arthralgia while 12 (75%) had also arthritis and/or rash. Incapacitating arthralgias were reported by 10 (62.5%) patients. Thirteen patients (81.3%) presented cardiovascular complications, mainly myocardial infarction (MI, 77%), as well as angina pectoris, heart arrhythmias, acute pulmonary edema and hypertension. The remaining patients presented renal and neurological complications (Guillain-Barré Syndrome and viral encephalitis). Of those initially diagnosed with a MI, one patient developed a cardiogenic shock, resulting in death. Patients were hospitalized on average for 12 days (range: 4-35). Thirteen (81.3%) patients had underlying diseases of which hypertension was the most common (56.3%) followed by diabetes (31%) and obesity (25%). The combination of hypertension and diabetes type II (n= 4; 25%) was the most common comorbidity combination. Detailed clinical data and further analysis on a bigger sample size will be presented. Knowledge on atypical presentations will improve early diagnosis and management of these patients avoiding possible life threatening conditions.

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PREDICTORS OF PLASMA LEAKAGE IN ADULT DENGUE PATIENTS

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It is estimated to have 390 million Dengue infections with 96 million cases worldwide. Mortality rate can be high ranging from 0.1% to 5%. Plasma leakage is the commonest complication in Dengue resulting Dengue Haemorrhagic Fever (DHF). The severity of leaking varies from individual to individual. Severe leaking can have a high morbidity and mortality if not detected and treated early. Therefore, it is important to detect plasma leakage early as such patients can be monitored carefully and fluid management can be manipulated according to the degree of leakage. Identifying predictors of plasma leakage would enable clinicians to closely monitor patients with such predictors to detect leaking early. Objective of this study was to determine predictors of plasma leaking in adult Dengue patients. All serologically confirmed Dengue patients admitted to Dengue Management Unit, National Institute of Infectious Diseases (formerly IDH), Angoda, Sri Lanka for four months from 1st of July 2014 were included in a prospective Case Control Study. Patients with plasma leakage were identified with serial ultra sound examination and were compared with others on predetermined parameters. There were 1000 patients with confirmed Dengue infection with 546 males and 454 females. Age ranged from 12 to 86 years. (mean 31 yrs.). 43.8% (n= 438) patients had plasma leakage (DHF) while 56.2% (n=562) did not have. There was no sex difference in patients with and without fluid leakage. Out of the warning signs in the WHO 2009 classification severe vomiting, abdominal pain and tenderness were significantly associated with plasma leakage ($p<0.05$) but not mucosal bleed or restlessness. In addition postural dizziness and platelet counts <50,000/microliter had higher risk ($p<0.05$) of developing plasma leakage as well as both overweight (BMI 23-27) and obesity (BMI >27). This study identifies several easily identifiable and observable parameters as predictors of plasma leakage in Dengue. These can be used identify patients likely to develop plasma leakage and to monitor them carefully to detect and to treat plasma leaking promptly thereby reducing morbidity and mortality.

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EARLY SEASON INCIDENCE, SUSCEPTIBILITY, AND WEATHER PREDICTS ANNUAL DENGUE HEMORRHAGIC FEVER INCIDENCE IN THAILAND

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Dengue is a mosquito-borne infectious disease that places an immense public health and economic burden upon Thailand. Annual outbreaks of varying sizes pose a particular challenge to the public health system because treatment of severe cases requires significant resources.

Accurately forecasting these outbreaks could help public health decision-makers implement and evaluate the efficacy of interventions. Here, we present a statistical model to predict annual dengue hemorrhagic fever (DHF) incidence for each Thai province in April using weather data and observed case counts through March. We used cross validation on data from 2000-2009 to select covariates a negative binomial generalized additive model. Two models - the model that performed best in cross validation and a model including only preseason incidence - were applied to data from 2010-2014 as if conducted in real time. We compared the results of these models to those of a baseline model that predicts the median incidence over the past ten years. The performance of each model varied across the administrative health regions of Thailand. The preseason incidence model performed best overall with better predictions than the baseline model in 63% of observed province years with a 22% reduction in absolute error, averaging across all province years. The best cross-validated model, including covariates for preseason incidence, estimated relative susceptibility, rainfall, temperature, and humidity made better predictions in Northern Thailand where weather and DHF incidence fluctuate from year-to-year. The baseline model had the best performance in Central Thailand, where annual DHF incidence is relatively stable. These results demonstrate that a combination of location-specific prediction models for dengue can aid public health decision-makers in assessing the potential risk of an epidemic in different geographic locations.

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SOCIAL CONNECTIONS AND CONTEXT IN DENGUE TRANSMISSION

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Dengue Virus (DENV) is a common arboviral disease in tropical and subtropical countries. Given the restricted range of mosquito movement (i.e., <100 m) and long-term immunity against the four DENV serotypes, human activities and herd immunity play critical roles in the spread of dengue. Specifically, individual activity spaces are socially constructed, and are shaped by activities (e.g., daily commuting, household tasks, social activities). Herd immunity is a limit on the number of potentially infectious hosts in a region and provides indirect protection for susceptible community members. In spite of a consensus on the importance of human activities and herd immunity, it is still difficult to accurately estimate their influence on dengue transmission. Epidemiologically significant measures of vector population density are also difficult to obtain. This uncertainty may be responsible for apparent conflicts in the findings of community-scale studies of DENV transmission. In this study agent-based models (ABM) have been used to jointly assess the effects of social networks, herd immunity, and vector density. Sensitivity analysis was used to understand the importance of social network attributes in terms of network type and number of social ties. The local context, in terms of herd immunity and mosquito density, significantly modulated sensitivity to social network specification. The results highlight the importance of characterizing host and vector population heterogeneity in studies of DENV transmission. Variation in model outcomes also helps us to understand factors that regulate the character of dengue transmission in terms of the overall intensity and focality of infections. The findings indicate that ABMs designed to test the effects of vaccination programs, where herd immunity is artificially increased, could be sensitive to the structure of social connections.

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ZIKA VIRUS INFECTION IN A COHORT STUDY TO ASSESS THE INCIDENCE OF DENGUE, STATE OF SÃO PAULO, BRAZIL, 2015, 2016

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Zika virus (ZIKV) was first identified in Brazil in 2015 by reverse-transcriptase polymerase-chain-reaction (RT-PCR) assays of serum specimens from patients in Northeastern Brazil, who presented with a dengue like illness that was characterized by rash, fever, myalgia, arthralgia, and conjunctivitis. Up to the 9th 2016 epidemiologic week, 22 Brazilian states notified ZIKV infection confirmed by PCR. In August 2014, in Araraquara, a city in the State of São Paulo, Southeastern Brazil, a prospective cohort for dengue surveillance was initiated through weekly phone calls to access the incidence of dengue among children and adolescents aged 2-16 years. 3,514 children and adolescents and their parents agreed to participate in the study and signed an Informed Consent Form. The follow-up showed 1,140 fever episodes and 314 confirmed dengue cases by laboratory diagnosis (NS1, qPCR IgM positive for dengue) in December 2015. PCR was performed for 492 children and adolescent negative for dengue, but with fewer and any sign or symptom, to investigate Zika virus circulations. The RNA isolated was subjected to a Real-Time PCR of a single step ("one-step") using TaqMan Fast Virus 1-Step Master Mix (ThermoFisher, Brazil) and the set of primers and probe as following: Primer Name Sequence SEQ 5' 3' 1086F CCGCTGCCCAACACAAG 1162R CCACTAACGTTCTTTTGCAGACAT 1107-FAM AGCCTACCTTGACAAGCAGTCAGACACTCAA In Araraquara, in 2015 occurred 1,804 dengue cases, being the largest epidemic of recent years. In 2016, 376 autochthones cases had already occurred up to now. In our cohort dengue cases appeared between November 2014 and August 2015 with a peak in April (117 cases). No case was positive for ZIKV in 2014 and 2015. In 2016, until the 16th epidemiologic week occurred 190 fever episodes with 9 dengue laboratory confirmed and 7 ZIKV confirmed by PCR in plasma with 2 also by urine. The most common symptoms were sore though, conjunctival hyperemia, headache, rash and pruritus. The two viruses are circulating in Araraquara, but it seems that there has been a susceptible depletion in relation to dengue with the concurrence of the emergence of the movement Zika virus.

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INAPPARENT DENGUE VIRUS INFECTION INCIDENCE, SAO PAULO, BRAZIL, 2014-2015

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Inapparent dengue virus infections have implications on the agent's transmission. They should be considered when evaluating control interventions such as vector control and vaccines. We have attempted to quantify the incidence of inapparent infections that occurred during the 2014 - 2015 dengue transmission season. A cohort study to assess the incidence of dengue among children and adolescents, from 2 to 16 years of age, in a previously recognized as a low endemic setting has started in August, 2014. A random sample of children and adolescents was selected from the population of Araraquara, a city in Central São Paulo State. Home visits were made to the families of selected children, to present the study and invite them to participate. The one who agreed to participate signed an Informed Consent Form. An interview on socio-

demographic characteristics was carried out and blood samples from the selected children were drawn for dengue baseline serology. Dengue IgG antibodies were tested by enzyme-linked immunosorbant assay (ELISA). Families are being contacted weekly for fever surveillance. Fever cases are submitted to dengue diagnosis tests. One year after recruitment a new blood sample was collected for IgG serology, in order to assess inapparent infections. The baseline seroprevalence of dengue IgG antibodies among the cohort participants was 15.3%. In the 2014/2015 dengue epidemic season the cumulative incidence of symptomatic laboratory confirmed dengue was 8.9%. The analysis of the one-year follow up samples is still ongoing. So far, results from 3,019 (85.9%) participants are available. Considering those who were seronegative for dengue at baseline and did not present symptomatic dengue infection, the seroprevalence after one year was 14.0% (320/2285), which may be interpreted as the incidence of inapparent infections. The rate inapparent/symptomatic dengue infections was estimated in 1: 1.6. It is lower than reported elsewhere. The sensitive suspect case definition (fever >37.5°C) may be responsible for this result. Unlike the incidence of symptomatic cases, the incidence of inapparent infections was not associated to age nor sex.

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DENGUE VIRAL INFECTION INDUCED CD95 EXPRESSIONS IN DIFFERENT B CELL SUBSETS

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Excessive expansion of antibody secreting cell (ASC) was observed during acute dengue viral infection. While ASC is directed against dengue viral antigen, a marked increase in plasma cytokine levels and soluble immune function molecules may drive these cells to become more susceptible to undergo apoptosis. Moreover, as previous studies showed that alterations of B cell subsets were observed by a remarkable decrease in naïve and memory B cells. It might be possible that these cells undergo apoptosis induction through interactions between Fas or CD95 expressed on these cells and Fas-L expressed by cross talking immature pDC or activated NK cells. In our study, peripheral blood samples from acute dengue viral infected patients were stained with fluorochrome conjugated monoclonal antibodies against CD3, CD14, CD19, CD20, CD21, CD27, CD38, CD45, CD95 and CD138. The frequency and density of CD95 expression in ASC and B cell subsets based on the expression of CD19, CD20, CD21, CD27, CD38 and CD138 were determined by flow cytometry. Results showed that CD95 expression was observed in all B cell subsets. However, the high levels of surface expression density were observed in plasmablast and memory B cell subsets. Interestingly, the frequency of naïve B cells that express CD95 was increased when compared to healthy subjects. Our study, therefore, provides important information on apoptosis induction of B cell subsets during acute dengue viral infection via the interaction between Fas and Fas-L. The results demonstrated that all B cell subpopulations, especially plasmablast, have a potential susceptibility to apoptosis through Fas signaling pathway. The study also suggested that a decrease in naïve B cells might be due to apoptosis induction in this subset.

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DENGUE DIVERSITY ACROSS SPATIAL AND TEMPORAL SCALES: LOCAL STRUCTURE AND THE IMPACT OF HOST POPULATION SIZE

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The micro-scale transmission dynamics of dengue ultimately determine who gets sick and the impact of interventions. Phylogeographic methods have been used to characterize pathogen dispersal at global and regional scales, but have yielded few insights into the local spatio-temporal structure of endemic transmission. Dengue virus infects >300 million people annually. Key mechanisms of introduction and maintenance remain unknown, including the number of distinct transmission chains that co-circulate within populations across different spatial scales. We geolocated 17,291 serotyped cases from Thailand from a 17 year period (1994-2010) and sequenced 800 viruses. We developed methods that compare the genetic similarity between viruses with the spatial distance between their locations to estimate the number of discrete transmission chains circulating within any area. We found that on average, 64% (95% CI: 41%-75%) of cases <200m apart in Bangkok were from the same chain (defined as having a common ancestor from the same dengue season) compared to 3% for cases <5km apart (95% CI: 1%-4%). Within 200m there were on average 1.7 transmission chains during a season, and with every 10-fold increase in population the number of chains increased 7-fold. However, there were significant heterogeneities across the city. Further, we found saturation in the number of chains circulating in equal sized areas at population densities >7,000/km2. We replicated these patterns using simulations where incidence is driven by local, density-dependent transmission; suggesting that ecological interactions in high-density environments limit the number of independent chains. We also found evidence for separation between national epidemics across Southeast Asia, suggesting minimal viral flow across borders. These results reveal hyper-local epidemics within a season and self-supporting dynamics within Thailand over the long-term. This study provides a framework for characterizing the number of independent transmission chains circulating within any community, with key implications for understanding local trends, including the impact of control efforts.

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AEDES ALBOPICTUS MIDGUT CELL LINE: A PRISTINE CELL LINE FOR *IN VITRO* STUDY OF ARBOVIRAL PATHOGENESIS

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The successful vaccine production for many arboviral diseases is hindered due to a very common problem i.e. non-availability of appropriate cell line, which mimics the microenvironment of the viral pathogenesis inside a host, providing an artificial cellular milieu for the virus to grow and multiply *in vitro*. Till date, many cell lines have been prepared from embryo, eggs and larvae of mosquitoes, which are extensively used for culturing arboviruses, but most of the arboviruses like Dengue virus, West Nile virus, Chikungunya virus, Japanese encephalitis virus, etc mainly infect the midgut of the mosquitoes; rendering available mosquito cell

lines inappropriate models for study of host-viral interactions. In this study, wild *Aedes albopictus* mosquitoes collected from different parts of Kolkata, India, were identified by an entomologist and were dissected to separate the midgut. Light microscopic and electron microscopic study of the cellular pattern on the mosquito midgut surface revealed the presence of stem cells, columnar cells, goblet cells and regenerative cells. Stained histological sections of midgut showed mucous coat enclosing the basal lamina on which the cells are embedded. Depending on the number of microvilli and microvilli-associated network, the posterior part of the midgut was targeted for preparation of the cell culture as it is the primary entry site for most of the arboviruses. The prepared cell culture was found stable without any lot to lot variation. The midgut cell culture was then challenged with dengue virus and then one alternative medicine "Rhus toxicodendron 6c" which is believed to be effective in dengue fever was tested for its efficacy as entry barrier and/or escape barrier in the primary cell line along with controls. Morphological changes were also analyzed. The medicine appeared to provide effective protection of the cell lines from dengue virus infection. Thus, the results of this study open up a new platform to study how arboviruses are normally able to surmount midgut infectivity barrier and midgut escape barrier, which will provide a better understanding of the innate host-viral interaction mechanism.

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A FULLY-HUMAN HYPERIMMUNE POLYCLONAL ANTIBODY PRODUCT FROM TRANSCHROMOSOMIC BOVINES TO TREAT DENGUE INFECTIONS

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No specific therapeutic is available to prevent or treat classical or severe dengue infections. Passive immunotherapy with target-specific immunoglobulin (IgG) is effective in the treatment of toxin, venom and pathogen mediated diseases. Current target-specific immunotherapeutics are either polyclonal IgGs, derived from human or animal plasma, or monoclonal antibodies. Each production approach has limitations. Human derived IgG (or convalescent plasma) require the recruitment of convalescent human donors in sufficient numbers. Monoclonal antibodies require lengthy development periods and pose the risk of escape mutants. Both these approaches can be prohibitively expensive. Animal derived heterotypic-antibody IgGs can cause severe reactions. Transchromosomic bovines (Tc-bovine) provide an alternative method to economically produce large quantities of target-specific IgG with fully-human antibodies. Tc-bovines have had their repertoire of bovine antibody genes deleted, and instead carry a human artificial chromosome containing the full repertoire of human antibody genes. Tc-bovines can rapidly produce up to 600 grams per month of hyperimmune, multi-pathogen, fully-human immunoglobulin (hIgG) to prevent and treat human diseases. Tc-bovines were hyperimmunized with psoralen inactivated tetravalent dengue vaccine (serotypes 1-4) and a purified Tc-bovine hIgG (SAB-123) with tetravalent dengue virus neutralization titers up to 2560 was produced. Four groups of Cynomolgous monkeys (n=3) infected with 5E5 PFU of dengue 1 on day 0 were infused with 100 mg/kg of SAB-123 on days -1 (prophylactic), +2 or +1&3 (therapeutic) or negative control hIgG on day -1. Control animals infused on day -1 all had detectable viremia on days 2-6. Those receiving SAB-123 on day -1 and day 1&3 had no detectable viremia at any time. Those receiving SAB-123 on day 2 only had detectable viremia for one day prior to transfusion. Similar hIgG therapeutic products for two separate infectious disease indications have obtained FDA clearance for clinical testing. These results warrant human evaluation of this product for treatment of dengue infections.

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CHARACTERIZATION OF CYD DENGUE VACCINE VIRUSES WITH HUMAN MONOCLONAL ANTIBODIES TARGETING KEY CONFORMATIONAL EPITOPES

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The Sanofi Pasteur CYD tetravalent dengue vaccine, which is now licensed in several dengue endemic countries, demonstrated significant efficacy in phase III studies with differences between serotypes. To characterize the quality and specificity of vaccine-induced antibody responses, we investigated the surface epitopes on each vaccine virus serotype using human monoclonal antibodies (MAbs). These antibodies have previously been shown to target conformational/quaternary epitopes, to be highly neutralizing, and either serotype-specific against DENV1 (1F4), DENV2 (2D22) or DENV3 (5J7) or cross-reactive against the 4 serotypes (1C19). A DEN4-specific antibody was not available at the time of the study. We monitored the binding of 1F4, 2D22, 5J7 and 1C19 with the vaccine monovalent lots included in Phase III clinical formulations using the following assays: Dot Blots, ELISA, Biacore® and PRNT. We used attenuated DENV as positive controls and some immature viruses or VLPs as negative controls for anti-DENV reactivity. Assays were set up and calibrated with cross-reactive anti-Envelope mouse monoclonal antibodies. In each assay, the CYD1, 2 and 3 vaccine viruses were found to be recognized by the MAbs with the expected specificity, and were strongly neutralized at levels comparable with those previously reported with wild type DENV. Biacore® further indicated a high functional affinity. Overall, these findings demonstrate that the CYD dengue vaccine viruses display key conformational and functional epitopes of wild type DENV. Future investigations will assess the neutralizing antibody response elicited by the CYD tetravalent vaccine against these critical epitopes, and how it relates with vaccine efficacy.

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PERIODICITY (LONG-TERM AND SHORT-TERM CYCLES) OF DENGUE IN VENEZUELA

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Mosquito-borne viruses are becoming major public health problems throughout the tropical and subtropical regions of the world. In Venezuela (South America), despite control measures, transmission of dengue virus (DENV) has become perennial with three large epidemics in the past decade. The long-term pattern of this disease has involved not only a general upward trend in cases but also a dramatic increase in the size of epidemic outbreaks. Previous studies indicate that DENV multiyear cycles are the result of both extrinsic (e.g. climate variability) and intrinsic (e.g. herd immunity and host susceptibility) factors. Here, we first explored the periodicity of dengue incidence in time-series of data (24 years) from several regions of Venezuela using wavelet analyses (WA), a statistical approach specifically developed for non-stationary patterns. Significant cycles of 1- to 3-year periods were identified. Additionally, we determined whether disease epidemics were related to local climate variability and regional climate anomalies such as the El Niño Southern Oscillation (ENSO) using WA to identify time- and frequency-specific associations. Understanding the periodicity of dengue in Venezuela can give useful insights about arbovirosis outbreaks. Indeed, the years 2014 and 2015 were marked by two relevant outbreaks of the emergent viruses:

chikungunya and zika. Therefore, our findings may be used to forecast dengue and other vector-borne viral epidemics and to improve disease surveillance and control strategies.

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USE OF THE DENGUE HUMAN CHALLENGE MODEL TO CHARACTERIZE THE ROLE OF HETEROTYPIC ANTIBODY IN PROTECTION AGAINST DENGUE INFECTION

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Dengue has become the most important mosquito-borne virus in the world resulting in nearly 400 million infections annually. There are four dengue virus (DENV) serotypes, each capable of causing the full spectrum of illness. This can range from an asymptomatic or mildly symptomatic infection to a life-threatening vascular leak syndrome. It is generally believed that infection with one DENV serotype confers long-lived protection against symptomatic re-infection with that same serotype but only short-lived (~3 months) protection against infection with a heterotypic DENV. We sought to test this paradigm using our controlled dengue human challenge model. Twenty-four flavivirus-naïve subjects were enrolled in a randomized, placebo-controlled, double-blind trial. The treatment assignments remained blinded until study day 270. Eighteen subjects received a trivalent mixture of the live attenuated candidate DENV vaccines rDEN1Δ30, rDEN3Δ30/31, and rDEN4Δα30. Eight subjects received placebo. Six months later, all returning subjects were given the DENV-2 challenge virus DEN2Δ30. Following receipt of the trivalent admixture 15 subjects developed rash (83%) and 11/18 (61%) had one or more DENV recovered from the blood. rDEN3Δ30/31 was recovered from 7 subjects, rDEN1Δ30 from 5 subjects, and rDEN4Δ30 from 2 subjects. Twenty-one subjects returned for challenge (15 trivalent recipients and 6 controls). Following challenge with DEN2Δ30, only 3 (20%) of those subjects who had received the trivalent mixture developed rash compared with 83% of the controls, indicating that the trivalent mixture imparted some protection against the challenge virus. The clinical, virologic, and serologic responses following administration of the trivalent admixture and following DENV-2 challenge will be presented. The role of heterotypic antibody and cellular immune responses will be discussed.

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LONGITUDINAL ANALYSIS OF B CELL RESPONSE TO INFECTION WITH A DENGUE-2 CHALLENGE VIRUS

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The four dengue virus (DENV) serotypes are the leading cause of arboviral disease globally, with 390 million new infections annually, and more than 40% of the world's population at risk. Both serotype-specific and cross-reactive antibody responses are observed at the population level, but the temporal patterns of how such antibodies emerge on an individual basis after primary DENV infection are not clear. A better understanding of the antibody response to primary infection can elucidate how circulating DENV-specific antibodies afford protection versus lead to risk of disease enhancement in secondary heterologous infection or vaccination. To address this, we analyzed the clonal evolution of B cells from the early plasmablast stage to the late memory stage in subjects from a controlled DENV human infection model. In a representative subject infected with DENV2 challenge virus we used Immune Repertoire Capture (IRC™) technology to identify over 400 unique, natively paired antibody heavy and light chains in the plasmablast repertoire. At six-month post challenge, we isolated and immortalized memory B cells from the same donor, and found that approximately 0.7% of IgG+ memory B cells exhibited reactivity to DENV. Most of the response was DENV2-specific though cross-reactive responses were also observed. Understanding the kinetics of the humoral response in this primary infection model will increase our understanding of the B cell evolution in response to DENV infection, and reveal insights on how specific vaccine components may be tailored immunogenically to maximize protective effect while minimizing risk.

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DENV PREEXISTING IMMUNITY EFFECT ON ZIKV INFECTION AND THE RELIABILITY OF DIAGNOSIS IN AN AREA WITH CO-CIRCULATION OF SEVERAL ARBOVIRUSES

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Previous studies have demonstrated the effect of cross-reactive sub-neutralizing antibodies in DENV severe clinical presentation. Since, DENV and ZIKA are closely related viruses, we tested whether there is any impact of previous DENV antibodies on ZIKV infection. To do this, we tested serum samples collected pre and post ZIKV epidemic and evaluate ZIKV neutralization capacity by PRNT50. On the other hand, there are several obstacles for accurate diagnosis of vector-borne viruses in endemic areas. The problem is significantly higher when several pathogens, with similar clinical characteristics, co-circulate in the same area. With the latest emergence of African arboviruses in South America, there is a need for reliable, cost-effective tools to help in differential diagnosis, especially in rural areas. Thus, we decided to compare the sensitivity and specificity of a rapid test for dengue virus infection (Dengue Duo-Antigen) versus a

molecular biology technique (qRT-PCR). We evaluated whether a particular test is better for differential diagnosis in an endemic region of Colombia where Dengue (DENV), Chikungunya (CHIV) and Zika (ZIKV) are often concurrently transmitted. We will show the sensitivity and specificity of qRT-PCR vs. Dengue Duo test, and discuss the impact of preexisting immunity on arbovirus transmission dynamics.

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VECTOR GENOTYPE INFLUENCES DENGUE VIRUS INTRA-HOST GENETIC DIVERSITY IN MOSQUITOES

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During infection of their arthropod vectors, arthropod-borne viruses (arboviruses) such as dengue viruses traverse several anatomical barriers that are believed to cause dramatic reductions in population size. Such population bottlenecks challenge the maintenance of viral genetic diversity, which is considered critical for fitness and adaptability of arboviruses. Anatomical barriers in the vector were previously associated with both maintenance of arboviral genetic diversity and alteration of the variant repertoire. However, the relative role of random processes and natural selection, and the influence of vector genetic heterogeneity have not been elucidated. In this study, we used high-throughput sequencing to monitor dengue virus genetic diversity during infection of several genetic backgrounds of their mosquito vector. Our results show that initial infection of the vector is randomly founded by only a few tens of individual virus genomes. The overall level of viral genetic diversity generated during infection was predominantly under purifying selection but differed significantly between mosquito genetic backgrounds. Thus, in addition to random evolutionary forces and the purging of deleterious mutations that shape dengue virus genetic diversity during vector infection, our results also point to a role for vector genetic factors in the genetic breadth of arbovirus populations.

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A CLOSER LOOK AT ADE AND OAS IN THE SECONDARY DENGUE PLASMABLAST RESPONSE

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Epidemiological studies have linked heterologous secondary DENV infections with increased disease severity, implicating pre-existing immunity in the form of antibody titers as a risk factor for severe disease. In this study, we focus on B cell responses generated during the acute phase of secondary DENV infection, and describe their potential to participate in antibody dependent enhancement (ADE) and original antigenic sin (OAS). We isolated plasmablasts from four Thai patients during ongoing DENV2 infection and generated 53 monoclonal antibodies by single-cell immunoglobulin gene expression. The antibodies were largely cross-reactive to two or more DENV serotypes, with a small subset exhibiting serotype-specific binding and neutralization activities *in vitro*. Interestingly, although all patients were infected with DENV2 at the time of the study, a majority of the antibodies generated from two patients displayed stronger neutralization of DENV1 than DENV2. These findings were echoed at the serum level, where a clear bias in neutralization was observed towards DENV1 compared to DENV2. This neutralization bias is strongly reminiscent of OAS. Additionally, a majority of DENV-neutralizing mAbs either moderately or potently enhanced DENV infection of U937 cells indicating that the potential for ADE is not limited to cross-reactive mAbs. Our studies provide basis for future work examining the impact of antibody responses on dengue immunopathology.

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DISPLAY OF QUATERNARY EPITOPES RECOGNIZED BY DENGUE VIRUS NEUTRALIZING ANTIBODIES

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Dengue virus (DENV) is the causative agent of dengue fever and dengue hemorrhagic fever. DENV and its mosquito vectors are widely distributed in tropical and subtropical regions and the disease is endemic in over 100 countries. Dengue vaccine development is challenging because of need to protect against four antigenically distinct DENV serotypes and evidence that, under some conditions, specific immunity to the virus can enhance disease. Recent studies have led to the identification of epitopes on the DENV envelope (E) protein targeted by human neutralizing antibodies. Some epitopes are preserved on the monomeric E protein, while other epitopes are complex and require the assembly of higher order E protein structures required for virion assembly. Here we describe studies to optimize the display of quaternary epitopes on artificial surfaces. The ectodomain of DENV E protein was expressed as a soluble recombinant protein (recE), which was secreted from cells. RecE was purified from the culture media and conjugated to a solid matrix. Using a large panel of human and mouse derived monoclonal antibodies, we confirmed that the conjugated protein was properly folded. Moreover, by adjusting factors such as pH, salinity and protein density, we optimized the display of quaternary structure neutralizing epitopes known to be critical for inducing protective antibody responses. These results have implications for developing novel subunit vaccines displaying quaternary epitopes from flaviviruses.

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RAPID ACTIVE SEROPREVALENCE SURVEYS AS A TOOL TO MEASURE DENGUE VIRUS DISEASE BURDEN IN RESOURCE-LIMITED SETTINGS

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Cost-effective surveillance systems capable of accurately detecting acute febrile illness (AFI) are necessary to evaluate the endemic burden of diseases such as dengue virus (DENV) and to estimate the potential effectiveness of vaccines. Cross-sectional seroprevalence surveys are commonly used for outbreak investigations, but they have not been validated as a timely, cost-effective alternative to active surveillance. We used a 2-stage cluster design (30 clusters of 7 households) to enroll children age 0-17 years in a rural, resource-limited region of Guatemala into two parallel surveillance systems to estimate the burden of AFI and DENV. In the prospective Participatory Syndromic Surveillance (PSS) arm, 207 households with 483 children (enrolled Apr-Sep 2015) were provided a wireless internet-connected smartphone with a symptom diary application and asked to submit weekly self-reports of fever. Subjects reporting 2+ days of fever were visited and offered DENV testing (PCR and IgM). In the Rapid Active cross-sectional Surveys (RAS), 377 children from 209 households (cycle 1), and 369 children from 210 households (cycle 2) from the same community were surveyed for self-reported fever within the preceding 7 days and offered testing for DENV by IgM regardless of symptoms, and by PCR if fever was present for 2+ days. In the PSS arm, 71 children reported AFI during 362 person-years of observation (19.6

cases/100 person-years), and 3 of 40 (8%) tested were DENV+. In RAS cycles 1 (Oct-Nov 2015) and 2 (Jan-Feb 2016), 74 (20%) and 53 (14%) children reported AFI in the preceding week and 3/13 (23%) and 6/29 (21%) tested were DENV+, respectively. In logistic regression models adjusted for sex, younger age was a significant predictor of AFI symptoms in the RAS cycles but not in the PSS subjects. Younger age was not associated with DENV+ AFI. Our data demonstrate a significant burden of AFI and DENV in the community. The more cost-effective RAS cross-sectional surveys provided more sensitive estimates of AFI incidence and DENV infection rates than the smartphone-based PSS active surveillance cohort, though further surveillance and data collection are needed.

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NATURAL AND LABORATORY-DERIVED GENETIC VARIATION IN DENGUE VIRUS TYPE 2 AT ENVELOPE PROTEIN POSITIONS 202 AND 203 MODULATES ANTIGENIC AND IMMUNOGENIC PROPERTIES

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The four dengue virus types (DENV1-4) cause up to 400 million infections each year. There is antigenic variation within and among the dengue types, but the genetic determinants of DENV antigenic variation are poorly understood. Others have shown that a single mutation at K204R (at the dimer interface of envelope [E] domain II) in DENV1 prototype strains drastically alters neutralization by a monoclonal antibody that targets a cryptic epitope exposed through virion breathing. Using antigenic cartography with primary infection African green monkey (AGM) antisera, we have found that a single amino acid substitution at a neighboring position, E202K, dramatically increases neutralization by homologous and heterologous antisera. Further, the AGM inoculated with E202K had a response highly focused to the homologous strain and remained the AGM's only detectable neutralization titer five months post-inoculation, while the AGM inoculated with the wild-type strain had balanced neutralization of diverse DENV2 strains. We are testing for differential neutralization of DENV2 E202K compared with the wild-type strain using monoclonal antibodies directed at cryptic epitopes as well as for improved neutralization with extended incubation times, suggestive of virion breathing. We also analyzed available E sequences in GenBank for natural variation and found that while positions 202 and 204 are highly conserved within and across serotypes, position 203 is naturally variable and differs between genotypes of DENV2 as well as DENV4. Interestingly, strains with 203D, including American genotype DENV2 strains shown by others to be cross-neutralized by primary DENV1 antisera, cluster closer to DENV1 antisera on antigenic maps, while Asian DENV2 genotypes with 203N are more distant from DENV1. We are testing if this antigenic difference is caused by exposure of a cryptic epitope. Understanding the mechanistic basis of the antigenic and immunogenic effects of genetic variation at 202-204 may provide insights into the antigenic representativeness of laboratory-adapted strains as well as if virion flexibility is important to DENV antigenic evolution.

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CO-INFECTION OF DENGUE VIRUS BY SEROTYPES 1 AND 2 IN A PATIENT FROM STUNG TRENG PROVINCE, CAMBODIA

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Dengue virus (DENV), the etiological agent for dengue fever and dengue hemorrhagic fever/dengue shock syndrome is a significant contributor to the morbidity and mortality rates in tropical and subtropical regions of the world, especially in Southeast Asia. There are currently four circulating clinical serotypes, and all of them have circulated in Cambodia during the past five years, with serotype1 (DENV-1) having historically been described as the predominating serotype and co-infection has not been commonly identified. Here, we describe a case of DEN-1 and DENV-2 co-infection that represents the first reported case of this serotype combination from Cambodia. The case was from Stung Treng Province, Cambodia, and was enrolled in a passive febrile surveillance study cohort administered by US Naval Medical Research Unit-2 (NAMRU-2). The case had classic dengue fever symptoms. Both dengue rapid test and serology were positive. Flavivirus screening was performed with Real Time-PCR and then confirmed as DENV-1 and DENV-2 co-infection by semi-nested PCR. The remainder of his hospital course was uncomplicated and recovered six weeks later without sequel, corroborating with previous reports. This case highlights the importance of dengue surveillance with serotyping. It demonstrates that dengue co-infection with different serotypes can occur naturally, and can go undetected if only rapid testing is performed. Absence of serotype-specific information in individual cases may impede clinical management, resulting in potentially unnecessary or detrimental treatment. With such high dengue incidence rates on national and regional levels, the availability of such information has substantial potential benefits to population health.

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MAPPING HUMAN NEUTRALIZING ANTIBODY RESPONSES TO DENGUE VIRUS SEROTYPE 4

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Dengue viruses (DENVs) are mosquito-borne flaviviruses consisting of four serotypes (DENV1-4). Primary DENV infections develop protective type-specific neutralizing antibodies, only to the serotype of exposure that target predominantly the quaternary structure epitopes. While specific quaternary epitopes responsible for the neutralization of DENV serotypes 1, 2 and 3 have been well defined, very little is known about the molecular basis of serotype 4 neutralization by human antibodies. This is a significant gap because a successful dengue vaccine has to induce protective antibodies to all 4 serotypes. We aimed to characterize the human long lived plasma cell (LLPC) and memory B-cell (MBC) derived neutralizing antibody responses in people exposed to primary DENV4 infections. To characterize the LLPC-derived responses, specific populations of antibodies were depleted from naturally infected DENV4 immune

subjects and DENV4 NIH monovalent vaccine recipients. In parallel, MBCs from naturally infected DENV4 immune subjects were transformed with Epstein Bar virus (EBV) to produce human hybridomas secreting DENV4-specific hMAbs. Two type-specific DENV4 neutralizing hMAbs were isolated and epitope mapped using binding and neutralization assays with wild type and recombinant DENVs. Further, shotgun mutagenesis studies aided in mapping the critical residues for these monoclonal antibodies. Antibody depletion studies showed that the DENV4 immune subjects had type-specific antibodies that strongly neutralized DENV4 only. These serum properties were also reflected in two hMAbs that strongly neutralized DENV4 only. The epitopes of the two DENV4 antibodies were mapped to the EDI/EDII hinge region. Importantly, LLPCs and MBCs in the subject from which the mAbs were isolated targeted the same epitope regions. A significant proportion of the type-specific responses induced in the NIH DENV4 monovalent vaccine recipients were also directed to the EDI/II region. We will discuss the specific location of the DENV4 neutralizing site and also the implications of our work for natural infection and dengue vaccine induced antibody responses.

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DETERMINING THE EFFICACY OF LARVIVOROUS FISH, COMMUNITY ENGAGEMENT, AND A NOVEL SLOW RELEASE PYRIPROXYFEN FORMULATION SUMILARV® 2MR ON DENGUE VECTORS (*AEDES AEGYPTI* AND *AEDES ALBOPICTUS*) IN CAMBODIA: A CLUSTER RANDOMIZED TRIAL

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Asia records 70 percent of the 390 million dengue infections occurring each year, and Cambodia has one of the highest per-capita incidence rates in the region. Due to the relatively high cost of the current vector control methods and documented insecticide resistance in Cambodia there is an urgent need to find alternative cost-effective solutions for *Aedes* vector control that are operationally feasible by the National Dengue Control Program. A cluster randomized, controlled superiority trial was developed to establish the effectiveness of an integrated vector management approach using guppy fish (*Poecilia reticulata*), a new slow release pyriproxyfen matrix (Sumilarv® 2MR), and community engagement through a clear Community for Behavioral Impact (COMBI) strategy. The trial based in Kampong Cham, Cambodia includes 30 clusters with approximately 200 households (1000 individuals) per cluster and runs from October 2015-October 2016. The clusters were randomly assigned with a 1:1:1 allocation through a public randomization process to one of three arms; (1) all interventions, (2) guppies and COMBI activities, and (3) control. The control area receives only interventions currently available through the government, which currently includes insecticide distribution and health education during outbreaks. To avoid spillover effects, clusters are at least 200 meters from the nearest household as *Aedes aegypti* in this region have an average flight range of 50-100m. The primary outcome is the population density of adult female *Aedes*, and will be evaluated through four entomological surveys. Secondary outcomes include classical Stegomyia indexes, coverage rates of the intervention, and changes in Knowledge, Attitudes, and Practices (KAP) indicators evaluated through monthly monitoring forms and baseline/endline KAP surveys. Polymerase chain reaction will be used to determine dengue virus rates in adult female *Aedes* mosquitoes. The results of the trial will be used to inform policy recommendations for Cambodia.

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DENGUE OUTBREAK IN EAST DELHI, DELHI STATE, INDIA, 2015

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Dengue has emerged as a major vector borne public health problem in India. It is endemic in capital city-state, Delhi, which experienced its largest ever dengue outbreak in 2015. An outbreak investigation was conducted in the East Delhi Municipal Corporation (EDMC) area with objectives to characterize outbreak features and to assess risk factors. A case of dengue was defined as "any person residing in EDMC area and had fever (2-7 days duration) between 14th May and 18th December, 2015 and tested positive for IgM ELISA or NS1 antigen ELISA". We prepared a line list and conducted descriptive analysis. We analyzed larval survey data. A 1:2 case control study was conducted to assess risk factors. Controls were persons from case's neighborhood with no fever history. We collected data on living conditions, domestic mosquito breeding sites, vector control and personal protection measures. There were 1775 dengue cases including 8 deaths. Age group 10 to 14 years (273 cases, 16.5%) most affected. Males were 1038 (61%). Median age was 21 years (Range: 7 months to 88yrs). Attack rate was 44 per 100,000 population. NS1 antigen ELISA test was positive in 1169(66%) cases. There was circulation of DENV 2 and 4 sero-types. In August, *Aedes* mosquito breeding detected in 14 per 1000 house visits and Breteau index was 15. Risk of dengue was more in those lived in overcrowded houses (OR 2.02, 95% CI 1.2-3.2), stored water in containers (OR 1.8, 95% CI 0.8-3.9), dumped waste disposables around/over the house (OR 1.6, 95% CI 0.7-3.6), spent day time in work place or school (OR 1.6, 95% CI 0.9-2.8) and low education (OR 1.4, 95% CI 0.8-2.3). Less risk was among those used larvicide in desert cooler (OR 0.3, 95% CI 0.08-1.1), worn trouser and full sleeved clothes (OR 0.6, 95%CI (0.4-1.1) and covered windows with mesh (OR 0.8, 95% CI 0.5-1.3). Factors like increased *Aedes* mosquito breeding in key domestic sites, circulation of multiple DENV sero-types, overcrowding and low education led to the outbreak. Concurrent anti-larval and anti-adult measures are needed to control *Aedes* mosquito. Active community participation to monitor key breeding sites and personal protection advocacy are needed.

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CORRELATION OF CLINICAL DIAGNOSIS AND DENGUE ASSAYS IN CAMBODIA OVER SIX YEARS

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Dengue fever is a flavivirus infection endemic to tropical regions, estimated to be responsible for 50-100 million infections annually. As a part of an ongoing febrile cohort study in Cambodia, febrile patients (>38C) presenting to regional health centers are enrolled and tested for a variety of febrile illnesses. During the period of Jan 2010-Feb 2016, specimens were tested for dengue with varying combinations of rapid test (NS1), PCR, or paired ELISA, with the follow-up performed at four weeks. Of the 19,823 enrolled patients, 1,572 were given a clinical diagnosis of dengue, but 4070 patients ultimately had at least one positive confirmatory test of NS1, PCR, acute IgM, or convalescent IgG. Positive results were obtained in 876 samples tested for NS1, 1272 for PCR, 1243 for acute IgM, and 2500 for convalescent IgG, resulting in 349 positive paired serologies. Clinical diagnosis had a sensitivity of 57.5% and specificity of 90.2%, but a PPV of 33.4 for correlation with NS1. NPV was 96.1. PCR performed similarly in relation to clinical diagnosis (Se 43.8%, Sp 94.8%, PPV 38.7, NPV 95.8; $p<0.001$). Analysis of a subset of 4667 patients for whom all tests were performed showed only marginal differences in comparison to the whole cohort. Clinical diagnosis performance decreased when used in

combination with the historical gold standard of paired serologies, with a sensitivity of 40.7%, specificity of 87.5%, and PPV of 17.1. NPV was 95.9 ($p<0.001$), and showed an overall positivity rate of 6.0%, as opposed to 7.3% for NS1 and 10.7% for conventional PCR. Distinct seasonality was observed for all measures. Serotyping was performed for 1271 samples. Predominance varied by year, but across the six years, serotype-1 was most common (62.3%), followed by serotype-2 (23.3%), type-4 (12.5%) and type 3 (1.9%). Dengue fever continues to be an illness with significant morbidity in Cambodia. Newer diagnostic tools such as rapid NS1 antigen tests and PCR should be used in resource-limited settings to supplement clinical diagnosis. Their improved accuracy may help reduce unnecessary antimicrobial use and improve quality of care.

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REGULATION OF PROTEIN TRANSLATION IN MOSQUITO CELLS INFECTED BY DENGUE 2 VIRUS

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Dengue virus (DENV) is naturally transmitted between humans by *Aedes* mosquitoes. The virus generally replicates and amplifies to a high level of virus progeny population in mosquito cells which usually survive the infection. As many other viruses, DENV can modulate host protein translational machinery that benefits for viral infection. In order to explore its regulatory mechanism, we applied a technique of SUnSET which is a nonradioactive measurement of protein synthesis in C6/36 cells with DENV-2 infection. The result revealed a significant shutdown of newly synthesized proteins in C6/36 cells infected by DENV-2. As UV-inactivated DENV-2, it seems that virus replication after entry is required to trigger signals for protein synthesis in mosquito cells. It has been reported that the initiation of cap-dependent translation is a key step during the process of protein synthesis via the assembled eIF4F complex which targets 5'-cap of mRNA, and may be hindered by the phosphorylation status of eIF4E-BP, a component of the eIF4F complex. Expression level and phosphorylation of eIF4E-BP has been observed to reduce in C6/36 cells with DENV-2 infection for 24 h. It suggested that the eIF4E-BP is one important factor involving in host cap-dependent translation of mosquito cells, particularly in the status of DENV-2 infection. On the other hand, PERK is a signaling pathway which may be triggered by DENV infection, causing attenuation of protein translation, in virus-infected cells. In this study, the PERK inhibitor (GSK2606414) was implemented to DENV-2-infected C6/36 cells, resulting in recovery of protein synthesis, implying that this signaling pathway was rather likely involved in modulating protein synthesis. However, it remains to be work out for understanding how these two factors involving in protein synthesis work together.

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RECENT SCIENTIFIC AND CLINICAL ADVANCES IN SANOFI PASTEUR'S DENGUE VACCINE PROGRAM

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The first dengue vaccine has been licensed by several dengue-endemic countries in Asia and Latin America for use in 9-45 or 9-60 year-olds. Licensure was supported by two pivotal phase 3 trials conducted in Asia, with 10,275 participants aged 2-14 years, and in Latin America, with 20,869 participants aged 9-16 years [NCT01373281 and NCT01374516 respectively]. Long-term safety follow-up studies, as recommended by the WHO, are currently ongoing and available data will be presented. Pooled efficacy results for the active surveillance period from both trials in subjects ≥ 9 years old demonstrated 65.6% (95%CI 60.7-69.9), 80.8% (70.1-87.7) and 93.2 (77.3;-98.0) efficacy against all symptomatic virologically-confirmed dengue (VCD) cases, hospitalized cases and severe dengue cases, respectively. During long-term follow-up in the third year of phase 3 and 2b trials, the pooled relative risk of hospitalized VCD cases among participants ≥ 9 years of age was 0.50 (0.28-0.89). In totality, these efficacy and safety data determined the currently licensed indication. Post-phase 3 investigations focusing on the quality of vaccine-induced responses: i) affinity and serotype-specific neutralization of antibodies, ii) infectivity and immunogenicity of the vaccine in a relevant *in vitro* tissue module (MIMIC), and iii) detection of key epitopes on the vaccine using clinically relevant human monoclonal antibodies, were initiated to understand the biological basis of these observed trial results. To complement findings, immune correlates derived from PRNT50 antibody neutralization data will be presented. Together, these data further support a vaccination strategy targeting high disease burden age ranges, combining routine vaccination with several catch-up cohorts at introduction would substantially reduce the burden of dengue disease in endemic regions.

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IMMUNITY TO ZIKV, DENV AND CHIKV IN A NON-ENDEMIC HUMAN IMMUNE COHORT IN PORTLAND, OREGON

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Zika (ZIKV), dengue (DENV) and chikungunya (CHIKV) are the most important emergent and epidemic mosquito-borne viruses worldwide. These viruses often share the same vectors and frequently co-circulate. The determinants of natural human immunity to these viruses are not fully characterized, nor are the degrees to which immunity to one cross-reacts with the other viruses. Moreover, their co-circulation potentially complicates traditional serological diagnoses and poses a particular challenge for safe and effective vaccine design. Cohorts of naturally immune individuals are vital resources for defining the critical correlates of ZIKV, DENV and CHIKV immunity. While international immune cohorts are often used to study natural arboviral immunity, local immune cohorts offer advantages over internationally based cohorts, including: 1) recruited individuals are unlikely to be confounded by repeat infection over study periods 2) adult recruits can provide large volume serum and cell samples serially that can be processed locally and contemporaneously, 3) given time, a local cohort is expected to include donors with diverse exposures that vary over time, geography, and viruses beyond what would be found at any single international site. Here we report demographic, travel, medical and virus exposure data, baseline serum neutralization values, cross-neutralizing activity between ZIKV, CHIKV and DENV immune sera against each other as well as West Nile Virus, Yellow Fever Virus and Japanese encephalitis virus for a human immune cohort in Portland, Oregon. We characterize persistence of neutralization over time, evaluate neutralizing antibody decay and report results of virus specific B-cell frequency. Long-term we expect this cohort to provide high-value immune sera and cells for both dissecting components of protective ZIKV, DENV and CHIKV immunity and validating candidate targets and correlates of long-term arboviral immunity.

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SPATIOTEMPORAL ANALYSES OF DENGUE HOSPITALIZATIONS CODED IN BRAZIL

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Dengue is the most rapidly spreading vector-borne viral disease and now a global health threat due to its presence in almost every tropical region and its alarming incidence increase within the last decade. Here, we have studied the temporal and regional epidemiological patterns of dengue in

Brazil in order to identify factors that were associated with higher numbers of hospitalizations from 1998 to 2013. We found that this number had increased in recent years, with a lower incidence in the southern part of the country. A marked seasonality was observed, with cases peaking during periods of the year in which mosquito abundance and activity were higher due to optimal levels of humidity and temperature. A novel contribution of our analyses is that the seasonality of dengue hospitalizations had a clear West-East gradient in Brazil. The analyses also revealed a higher proportion of children that were hospitalized due to dengue within the last 15 years, especially during strong outbreaks. These changes are likely to be a result of multiple factors, such as the accumulation of multitypic immunity in adults during the 20 years following re-introduction of dengue virus into Brazil in 1986 and thus the higher probability for children to be susceptible or monotypically immune, and the re-emergence of the more aggressive dengue virus strain DENV2 in 1990. Alternative explanations for the higher number of dengue outbreaks and hospitalizations and the higher proportion of children affected are fluctuations in serotype-specific transmission intensity, regional variations in circulating DENV serotypes, and the density of the vector population. Based on these results, we may speculate that the number of children hospitalized due to dengue is likely to increase in the southern part of the country within the next years. Our findings may allow health systems to improve control interventions and contribute to reducing dengue morbidity and mortality by using integrated vector control in conjunction with early diagnosis.

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HYPOXIA ENHANCES ANTIBODY-DEPENDENT DENGUE

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Dengue virus (DENV) has been found to replicate in lymphoid organs, such as lymph nodes and spleen, as well as the liver in post mortem examination. These organs universally have significantly lower oxygen levels (lymphoid organs~0.5-4.5% O_2) compared to atmospheric air (~20% O_2) due to the vascular anatomy. How physiological oxygen levels in the organs where DENV replicates, affect DENV infection through hypoxia-induced changes in the immune response has, however, never been investigated. We report here that, compared to cells cultured at 20% O_2, infection of THP1 and primary monocytes at 3% O_2 (hypoxia) required 4-fold more antibody for complete neutralization. Furthermore, sub-neutralizing levels of antibodies produced 2-3 fold higher enhancement in DENV infection under hypoxic conditions. We show that these observations were mediated by the hypoxia-induced upregulation of FcγRIIA but not FcγRIIB expression. High-resolution microscopy shows that FcγRIIA directly mediates internalization of DENV immune complexes under hypoxic conditions. Mechanistically, the stabilization of hypoxia inducible factor (HIF1α) by hypoxia or chemically with desferrioxamine (DFX) under 20% O_2 conditions both upregulated FcγRIIA. However, DFX induced FcγRIIA expression only resulted in increased DENV immune complex attachment but not internalization into cells. In addition to the upregulation of FcγRIIA, a hypoxia driven but HIF1α independent increase in membrane ether lipid concentrations is required to synergistically increase internalization of DENV immune complexes for enhanced infection. Our findings thus indicate that the increased viral burden associated with secondary DENV infection is thus antibody-dependent but hypoxia-mediated and suggest a role for targeting hypoxia-induced factor for anti-dengue therapy.

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VIREMIA AT PRESENTATION IS ASSOCIATED WITH LONG-LIVED ANTIBODY RESPONSES TO DENGUE VIRUS

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Infection with one of four related dengue virus (DENV) serotypes is thought to elicit life-long, serotype-specific immunity but only short-lived immunity to the remaining 3 serotypes. This theory has been derived from the study of typical, symptomatic DENV infections. In the current study, we evaluated the humoral immune response to DENV infections that were detected in febrile children clinically diagnosed with a non-dengue illness (referred to here as atypical cases). 2,892 acute-phase serum samples were tested for DENV by real-time RT-PCR (rRT-PCR), and viremia was quantitated using a serotype-specific DENV multiplex rRT-PCR. Samples were collected as part of an ongoing pediatric dengue cohort study in Managua, Nicaragua. As part of the study, patients had healthy annual serum samples tested using a specific Inhibition ELISA to detect total anti-DENV antibodies. These data were used as a measure of the long-term humoral immune response to DENV. 130 atypical cases tested positive for DENV, and 111 had Inhibition ELISA results from paired pre- and post-infection annual samples. 53 cases (47.8%) showed seroconversion or a >4-fold increase in titer in pre- vs post-infection samples (referred to as a positive Inhibition ELISA result), which was significantly lower than expected based on data from typical, symptomatic cases (79.7%; $p<0.01$). Viremia at presentation was significantly higher in atypical cases with positive Inhibition ELISA compared to cases with negative Inhibition ELISA [mean 7.6 (SD 1.5) vs 4.5 (SD 1.6) log10 copies/mL serum, respectively; $p<0.01$), and this remained significant in multivariable analysis ($p<0.01$). Atypical cases also appeared to alter the response to subsequent (secondary) typical, symptomatic cases. All 16 patients with two typical cases developed the expected rise in Inhibition ELISA titer following a second DENV infection (reciprocal titer ≥ 2560). However, in patients with an atypical primary case, only 1/8 patients developed the expected response ($p<0.01$). These data have important implications for understanding DENV immunology, estimating DENV incidence, and modeling virus transmission.

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CHARACTERIZATION OF THE OVERWINTERING PROCESS OF JAPANESE ENCEPHALITIS VIRUS IN *CULEX* SPECIES MOSQUITOES

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Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus endemic in the Asia-Pacific region and has been continuously regarded as a potential threat to human and veterinary public health in North America. Previously, we and others have identified potential competent vector species and amplification hosts of JEV in North America. These studies suggest that the establishment of enzootic transmission is highly likely in the event of its

introduction. A significant gap in determining the likelihood of establishing endemic transmission of JEV in North America is whether or not the virus can successfully overwinter in persistently infected animals. As observed with West Nile virus, successful overwintering allows the initiation of transmission in the spring and would ultimately lead to its establishment in North America. Whilst persistent infection of amplification hosts can serve as one mechanism for overwintering, persistently infected arthropods are also considered an important mechanism for overwintering. In this study, the overwintering process was investigated by maintaining orally infected American mosquitoes species at 16°C. Infectious virus was successfully recovered from infected mosquitoes demonstrating that JEV can lead to persistent infection at lower extrinsic temperatures. Therefore, we conclude that infection of arthropod vectors can serve as a potential overwintering mechanism for JEV in the event of its introduction in to the United States.

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PROPOSED GUIDELINES FOR ADMINISTERING LIVE YELLOW FEVER VACCINE TO TRAVELERS

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The risk of acquiring yellow fever (YF) remains high in large parts of sub-Saharan Africa and South America. Travelers to endemic areas should expect that one in seven infected individuals will become symptomatic and 20 to 60% of symptomatic persons will succumb. Effective antivirals are not available. The principal control mechanisms involve mosquitoes, principally *Aedes aegypti* in the case of urban YF, and administration of the effective YF vaccine. The development of large urban slums in tropical areas facilitates the growth of *Ae. aegypti* in water filled containers making mosquito control difficult. In 2001 recognition of the rare possibility of vaccine-associated severe viscerotropic disease (YEL-AVD) with a case fatality rate of ~63% has made it necessary to consider the relative risks of acquiring YF and developing YEL-AVD. New guidelines for the administration of the live YF vaccine have been proposed. The guidelines begin with a risk assessment of prospective vaccinees in established groups associated with increased susceptibility to YEL-AVD: Males older than 55, women between the ages of 19 and 34 living in non-endemic areas of Peru, people of either gender older than 76, persons with a variety of autoimmune diseases and patients with thymomas. The risk assessment is then used in connection with an estimate of the risk of acquiring YF in the area of intended travel. The risk for a particular area is influenced by continent (the risk in Africa being substantially greater than the risk in S. America), current reports of YF activity and rainfall. In some instances in areas of S. America the risk of YEL-AVD may be equal or greater than the risk of YF. In situations in which the risks of both entities are high, the recommendation is don't travel. Although currently available information from official sources may be uninformative, judicious use of weather and map websites may be surprisingly helpful even for isolated rural communities in Africa and S. America. The decision to vaccinate with the live YF vaccine has become more complex, but the vaccine remains the most significant method for prevention of YF.

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COMPREHENSIVE MUTAGENESIS OF HCV E1/E2 ENVELOPE TO EPITOPE MAP ANTI-ENV ANTIBODIES AND FUNCTIONAL RESIDUES CRITICAL FOR HCV INFECTIVITY

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To obtain epitope maps for anti-HCV Envelope (E1/E2) monoclonal antibodies (MAbs), we individually mutated 552 residues of HCV (H77 strain) E1/E2 to alanine. Each mutant was expressed in human cells and analyzed for its effects on MAb reactivity. This 'Shotgun Mutagenesis' approach offers the capability of mapping both linear and conformational epitopes, even for structurally complex proteins such as the oligomeric and glycosylated HCV Envelope protein. This approach identified critical amino acids required for the binding of dozens of MAbs, and has also been used to propose E2 disulfide bond cysteine pairs that are not resolved by the available E2 crystal structures. This approach has helped define the range of immunodominant structures on HCV E1/E2 and identify novel neutralizing antibody epitopes that can be used for the development of improved therapeutics, diagnostics, and vaccine candidates. In addition, to identify residues important for HCV infectivity we produced infectious HCV pseudoviruses from each mutant Env clone in the library. These pseudoviruses were used to evaluate each Env clone for infectivity on target cells. This allowed us to identify critical E1/E2 residues whose mutation eliminated HCV infectivity, identifying crucial HCV E1/E2 structural components that enable HCV infectivity.

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ANALYZING THE IMMUNE RESPONSE TO ZIKA VIRUS: A REPORTER VIRUS PARTICLE (RVP) SYSTEM FOR SERUM AND ANTIBODY NEUTRALIZATION ASSAYS, AND A COMPREHENSIVE ALA-SCAN MUTATION LIBRARY OF ZIKV PRM/E TO EPITOPE MAP ANTI-ZIKV ANTIBODIES

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We have developed a reporter system, using pseudoinfectious reporter virus particles (RVPs), to facilitate analyses of the immune response to Zika virus (ZIKV) infection and anti-ZIKV vaccines. RVP-based assays provide a rapid reliable alternative to PRNT-based assays and are particularly suitable for high throughput screens of large panels of patient sera or isolated MAbs. ZIKV RVPs are replication-incompetent virus particles, antigenically equivalent to live viruses, containing the ZIKV prM and E envelope proteins, capsid protein, and a sub-genomic replicon encoding a reporter protein (luciferase or GFP). After infecting permissive cells, RVPs express their reporter protein, providing a convenient and reproducible quantitative assay for measuring the neutralizing capabilities of serum or individual monoclonal antibodies (MAbs). To further characterize the immune response to ZIKV infection, we have also developed a high-throughput strategy that enables the rapid identification of both linear and conformational antibody epitopes on ZIKV prM/E envelope proteins. We used Shotgun Mutagenesis technology to create a comprehensive library of 660 single mutations in ZIKV prM/E. The individual mutant expression plasmids were arrayed into 384 well plates and transfected into human cells to achieve native protein expression and folding. The immunoreactivity of MAbs to the prM/E variant in each individual well was quantified by high-throughput flow cytometry, enabling us to map a number of anti-ZIKV MAbs. The epitopes obtained are being correlated with MAb abilities to neutralize ZIKV *in vitro* and to protect against ZIKV infection *in vivo*.

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EVALUATION OF SELECTED REAL-TIME RT-PCR PROTOCOLS AIMING AT THE BEST POSSIBLE MOLECULAR DIAGNOSIS OF ZIKA VIRUS INFECTIONS

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Zika is an emerging infectious disease resulting from Zika virus (ZIKV) infections and usually presenting itself by dengue-like symptoms. Clinical manifestations of Zika, dengue and chikungunya are somewhat similar, making it difficult to reach the diagnosis based only on clinical grounds. To make the diagnosis even more difficult, there is an intense cross-

reactivity between Zika and other flavivirus antibodies. Thus, the correct diagnosis of Zika is better achieved by molecular methods, but due to the short-lived and low titer viremia, molecular methods need to have a high analytic sensitivity. Real-time RT-PCR (rRT-PCR) is the best diagnostic approach available since it is possible to design protocols with no cross-reactivity with other flaviviruses. In this study, the analytic sensitivity of some published probe-based rRT-PCR protocols to detect ZIKV genome was evaluated. Ten-fold serial dilutions of ZikaSPH2015 strain, titrated in Vero cells, with a final virus concentration ranging from 1000000 to 1 virus/dilution was spiked in the serum of a flavivirus naïve healthy donor. ZIKV RNA from all dilutions, as well as from serum and urine samples obtained from patients, was extracted and amplified by rRT-PCR with five different pairs of primers (ZIKVA, B, C, D and E) and their respective probe. The analytic sensitivity of each rRT-PCR protocol was evaluated by the cycle threshold (Ct), where the lower the value the higher the sensitivity. Among all rRT-PCR protocols, ZIKVD and ZIKVC had the lowest and highest Ct values, respectively. Besides, ZIKVC detected only to 1000 copies/µL, while the other primers could detect virus in all dilutions. ZIKVD also had the best sensitivity when using patients' samples. ZIKVD rRT-PCR protocol consistently detected ZIKV genome in serum and urine samples while ZIKVA and ZIKVB were more reliable in urine, and ZIKVC and ZIKVE were usually negative in any sample. In conclusion, ZIKVD rRT-PCR is the best protocol to detect ZIKV genome in any sample. The presence of possible mismatches between the ZikaSPH2015 strain and the sequence used by Tappe et al to design ZIKVC primers might be the cause of its low sensitivity.

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WHOLE GENOME SEQUENCING AND PHYLOGENETIC ANALYSIS OF ZIKA VIRUS ISOLATED IN INDONESIA

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Zika virus (ZIKV) was isolated from serum of a febrile patient in Jambi municipality, Sumatra, Indonesia during an outbreak of dengue-like illness in 2014. Although human ZIKV infections are normally associated with mild illness, WHO has declared the ZIKV outbreak a global public health emergency because of its strong association with Guillain-Barré syndrome and microcephaly. To understand the ZIKV evolution and its genetic characteristics, whole genome sequencing and phylogenetic analysis was performed on the ZIKV isolate (JMB-185). ZIKV RNA was extracted from cell culture supernatant and subjected to sequencing employing the Ion-Torrent Next-Generation Sequencing technology. The complete genome sequence of JMB-185 was assembled and aligned with all available ZIKV complete genome sequences retrieved from GenBank. To evaluate the evolutionary history of this isolate, we performed phylogenetic analysis using the Bayesian MCMC inference method. The molecular clock phylogeny analysis shows that the ZIKV JMB-185 strain, which shares common ancestry and time to the most recent common ancestor (TMRCA) with 2014 and 2013 Thailand strains around year 2008, has older lineage than the Polynesian and the Brazilian strains linked with microcephaly and is not closely related to those strains. The TMRCA result indicates that the ZIKV Jambi JMB-185 strain may have been in circulation in the South East Asia region, including Indonesia since 2008. We observed high nucleotide sequence identity and similarity between Indonesia, Thailand, and American strains. Further analysis including those of amino acid substitutions is in progress.

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CLINICAL CHARACTERIZATION OF ACUTE ZIKA PATIENTS IN BRAZIL, VENEZUELA, AND EL SALVADOR IN 2015/16

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Latin America and the Caribean are currently facing an epidemic of three co-occuring arboviruses - Dengue, Chikungunya, and Zika. The multi-center observational IDAMS study is one of the few prospective studies capturing incident zika cases alongside with dengue and chikungunya. The study was initiated in 2011 and patients ≥ 5 years with undifferentiated febrile disease within the first 72 hours were enrolled in three sites in Brazil, Venezuela, and El Salvador. In 2015, 574 patients were recruited in total, among them 334 in Brazil (194 in Recife, 55 in Fortaleza, 85 in Rio de Janeiro), 72 in Venezuela, and 168 in El Salvador. The aims of the IDAMS study are to evaluate risk factors for severe dengue disease and validate the case definition for presumptive dengue in the absence of confirmatory laboratory testing. A broad range of clinical signs and symptoms and laboratory values are assessed daily during the acute illness, and at follow-up 1 week later. The study is currently ongoing and the inclusion criteria were adapted to presence of fever and/or rash in 2016. PCR for dengue , zika (and possibly chikungunya) viruses will be performed on all samples from 2015 and 2016, following strict protocols. Dengue is diagnosed by an algorithm including PCR, NS1, and IgM seroconversion. Preliminary results show a considerable proportion of zika-PCR-positive cases. We will describe the spectrum of clinical manifestations in zika patients and analyze clinical and laboratory parameters associated with zika vs. dengue at enrolment and over the course of the disease. We will carry out multivariable regression, stratified by age group, day of illness, and country, and assess the heterogeneity between the sites before pooling the data. Results to be presented will include patients recruited up to June 2016 and are expected to have considerable impact on the validation of the zika interim case definition issued by WHO.

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DETECTION AND DIFFERENTIATION OF IGM RESPONSE TO RECENT EXPOSURE TO ZIKA AND OTHER VECTOR BORNE VIRUSES USING A MULTIPLEXED, BEAD BASED SEROLOGY ASSAY

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Since May 2015, South and Central America have experienced an epidemic outbreak of Zika Virus. Several travel related cases have already been identified in the United States. Zika infection during pregnancy has been implicated in the development of microcephaly in the developing fetus and is thought to be responsible for an increased incidence of Guillain-Barre syndrome. The expanding footprint of the disease, along with the devastating neurological effects associated with Zika Virus warrant a cost effective and specific diagnostic. In Zika infection, the detection of viral RNA in serum is transient and is only detectable for a few days post infection. Therefore, specific detection of IgM representing a recent infection from a vector borne virus as a companion to molecular detection is required in order to expand the diagnostic window. In areas

with co-circulating vector borne viruses, serological responses must not only be detected, but differentiated in order to provide accurate results. An effective test also must distinguish a recent infection from historical infections and vaccinated individuals. A serology based, multiplex microsphere assay was developed to detect and differentiate IgM response to Zika Virus, Chikungunya Virus, Dengue Virus (Serotypes 1-4), West Nile Virus, Yellow Fever Virus, Japanese Encephalitis Virus, Tick Borne Encephalitis Virus, St. Louis Encephalitis Virus, and Usustu Virus. Assay performance was established using clinical samples. The assay demonstrates required analytical specificity and is able to detect and differentiate each virus. The multiplex serological assay eliminates the need for sequential testing and complicated patient care algorithms. The multiplex format allows for simultaneous identification of IgM responses to a group of viruses that have historically been difficult to distinguish due to strong immunological cross reactivity and challenges with distinguishing old verses new infections.

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MAPPING THE GLOBAL DISTRIBUTION OF YELLOW FEVER

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The spread and recent outbreaks of Zika, dengue, yellow fever and chikungunya viruses across the globe in 2015/16, highlight the need to reassess our understanding of these arboviruses, including their current distributions and potential for spread into new areas. Yellow fever is vaccine preventable, yet incomplete coverage means that it is widely distributed in the tropics of South America and Africa where infections cause an estimated 29,000 to 60,000 deaths annually. Epidemiologists have long been concerned about the introduction of yellow fever into large urban populations of naïve individuals where it can spread rapidly from human-to-human transmitted by *Aedes aegypti* mosquitoes. Earlier this year, the confirmation of yellow fever cases, imported from an outbreak in Angola, in areas of China with established vector populations, coupled with the depletion of vaccine stockpiles, raised concerns that yellow fever could gain an uncontrollable foothold in Asia. The disease has been conspicuously absent from Asia to date, despite multiple opportunities for introduction and the apparent presence of all components of a suitable transmission cycle, but no barriers to its introduction have been identified so vigilance is vital. We produced high resolution evidenced-based maps of the current distribution of yellow fever in Africa and the Americas. These maps were derived from a boosted regression trees model that used geo-positioned data on occurrences of yellow fever infection, environmental and socio-economic variables, as well as vaccination coverage data. The outputs were then used to predict areas of suitability for yellow fever transmission in South and South East Asia. This work furthers our understanding of the current global distribution of yellow fever and the potential for its spread in parts of Asia.

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ACUTE ENCEPHALITIS SYNDROME IN ASSAM, INDIA: IMPORTANCE OF JAPANESE ENCEPHALITIS IN THE ADULT POPULATION, 2014-2015

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In India, thousands of cases of acute encephalitis syndrome (AES) are reported each year, predominantly among children. Japanese encephalitis virus (JEV) accounts for 13-18% of reported AES. From 2011-2015, Assam, a highly affected northeastern state, has reported over 1,000 AES cases annually. We conducted facility-based surveillance at Assam Medical College (AMC) hospital to characterize illness and evaluate potential etiologies of disease. Between Jan 2014-Dec 2015, serum and cerebrospinal fluid (CSF) samples were collected from each patient admitted to AMC with AES, defined as acute onset fever (>38°C) and ≥1 of altered mental status or seizures. Serum and CSF were tested for JEV IgM antibodies. A diagnosis of JEV was confirmed if JEV IgM was detected in CSF and probable if JEV IgM was detected in serum only. JEV-negative patients were evaluated for scrub typhus IgM, dengue virus IgM, and West Nile Virus (WNV) IgM in serum, and for bacterial/viral nucleic acids for *Streptococcus pneumoniae*, *Hemophilus influenzae*, and herpes simplex virus-1 (HSV) in CSF. Of 925 patients admitted with AES, 606 (66%) were male and 491 (53%) were ≥15 years old. CSF was collected from 772 (83%) patients and serum from 772 (83%). Among the 925, 329 (36%) were diagnosed with either confirmed (254, 28%) or probable (75, 8%) JEV. Of these 329, 174 (53%) were ≥15 years old, and 52 (16%) died. Among 596 JEV-negative patients, the following additional pathogens were detected in serum: scrub typhus IgM (67/485, 14%), dengue IgM (17/408, 4%). WNV IgM was not detected in any patient. CSF molecular testing in 254 patients indicated evidence of *S. pneumoniae* in 4 (2%) and *H. influenzae* in 2 (1%). Of 351 evaluated, HSV was detected in 6 (2%). JEV is the most common cause of AES in Assam, is associated with high mortality, and disproportionately affects adults, highlighting the need for ongoing adult JEV vaccination efforts in the region. The identification of additional treatable etiologies of illness, such as scrub typhus and pyogenic meningitis, has important clinical management implications, and underscores the need to employ a standardized laboratory testing algorithm for AES.

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HOSPITALIZATIONS BY HEPATITIS C BETWEEN 1998 AND 2013 IN BRAZIL: AN INTRIGUING AMAZONIAN TALE

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Hepatitis C (HCV) infection is a public health problem of global dimensions, affecting 3% of the world' s population. In Brazil, between 2.5% and 4.9% of the population is infected with HCV. The Brazilian Amazon region has been reported to be hyper endemic for HCV and two other hepatotropic viruses (Hepatitis B and D). Here we explore the temporal patterns of incidence of hospitalizations attributed to HCV in Brazil per state and age group. We used hospitalization data from 1998 to 2013, from the nationwide administrative database of the Unified Health System (SUS). The records of hospitalizations coded as Hepatitis C were filtered using the codes B171 and B182 and aggregated in monthly time-series by state and age groups. Incidences were obtained using census-interpolated matrices. Visual inspection and spatio-temporal parameters were obtained using the analytical software epipoi. Between 1998 and 2013, 24210 patients were hospitalized with a principal diagnosis of HCV infection. As expected, the majority of new HCV cases (12038 patients) were recorded in Sao Paolo that has an estimated population of 44.39 million people. However, the incidence rate of HCV-related hospitalization was significantly higher in the region of the state of the Acre (mean incidence rate > 0.12 and linear trend < 16x10^-4) compared to all other Brazilian states. A total of 340 hepatitis C cases were recorded in the SUS database, the majority of which (186 cases) aged between 40 and 59 years. We detected worrying high incidence rates of HCV hospitalizations in the Western Brazilian Amazon Region. An increased prevalence of HCV among health care workers in Rio Branco located at the State of the Acre has been previously reported (Parana J *et al*, Am J Trop Med Hyg, 2007). If it is ruled out that the detection of HCV is exceptionally better in this region, then the infection control services in the hospitals of the State of

the Acre need to be urgently evaluated. Our findings indicate the need for the implementation epidemiological awareness, prevention and control programs for Hepatitis C in this region.

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DESIGN AND VALIDATION OF A RAPID ASSAY FOR ZIKA VIRUS IN BIOFLUIDS AND INSECT VECTORS

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Understanding the dynamics of Zika virus transmission and formulating rational strategies for its control require diagnostic tools that are appropriate for resource-poor environments. We have developed a rapid and sensitive loop-mediated isothermal amplification (LAMP) assay that is highly specific for the Puerto Rican Zika virus isolate, PRVABC59, and related isolates in the Asian clade. The assay does not detect Senegalese or Ugandan Zika isolates, or dengue, yellow fever, West Nile, or chikungunya viruses. The conditions described for the PRVABC59-LAMP assay allow direct detection of virus in infected cells, mosquitos, serum and blood without reverse transcription or RNA isolation. Time to detection of a single infectious particle in blood is 60 minutes in laboratory or field settings. It offers rapid, specific, sensitive and inexpensive detection of the Zika viruses currently circulating in the western hemisphere. Results from this assay will be presented.

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MAPPING GLOBAL SEASONAL SUITABILITY FOR ZIKA VIRUS TRANSMISSION

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Zika is a zoonotic mosquito-borne disease that became a global public health emergency in late 2015 when it spread throughout the Americas. While genetically similar to other globally distributed arboviruses such as dengue and chikungunya, Zika is of particular concern due to its link with neurological birth defects among infected pregnant mothers. Given this potential threat and the alarming rate of spread of the virus global predictions are urgently needed to assess where and when the virus may spread next with enough warning to deploy surveillance, control and diagnostic measures to protect those most at risk. Key insights into Zika seasonality can be gained through analysing the seasonal drivers of its principle mosquito vectors: *Ae. aegypti* and *Ae. albopictus* and their ability to transmit viruses. Here we combine habitat suitability analyses of these mosquito species with viral incubation period models to produce a series of global maps that depict when the arboviral season begins and ends around the world. This revealed broad areas of potential Zika transmission in temperate latitudes for long periods of the year, while also showing that the outbreak in some parts of South America may have been prematurely ended due to seasonal forcing instead of depletion of susceptible hosts. This could mean some populations in South America could potentially see a return of Zika sooner than previously estimated. The resulting predictions can be used for real time prediction of risk, better understanding past outbreaks and preparing appropriately for future Zika outbreaks.

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WORKING WITH ZIKA AND USUTU VIRUSES *IN VITRO*

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Usutu (USUV) and Zika (ZIKV) viruses are emerging arboviruses of significant medical and veterinary importance. These viruses have not been studied as well as other medically important arboviruses such as West Nile (WNV), dengue (DENV), or chikungunya (CHIKV) viruses. As such, information regarding the behavior of ZIKV and USUV viruses in the laboratory is dated. Usutu virus re-emerged in Austria in 2001 and has since spread throughout the European and Asian continents causing significant mortality among birds. Zika virus has recently appeared in the Americas and has exhibited unique characteristics of pathogenesis, including birth defects and transmission. Information about the characteristics of USUV and ZIKA viruses are needed to better understand the transmission, dispersal, and adaptation of these viruses in new environments. Since their initial characterization in the middle of last century, technologies and reagents have been developed that could enhance our abilities to study these pathogens. Currently, standard laboratory methods for these viruses are limited to 2-3 cell lines and many assays take several days to generate meaningful data. The goal of this study was to characterize these viruses in cell culture to provide some basic parameters to further their study. Cell lines from 17 species were permissive to both ZIKA and USUV. These viruses were able to replicate to significant titers in most of the cell lines tested. Moreover, cytopathic effects were observed in 8 of the cell lines tested. These data indicate that a variety of cell lines can be used to study ZIKA and USUV and may provide an updated foundation for the study of host-pathogen interactions, model development, and the development of therapeutics.

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PREDICTING INTENSITIES OF ZIKA INFECTION AND MICROCEPHALY USING TRANSMISSION INTENSITIES OF OTHER ARBOVIRUSES

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The World Health Organization has declared Zika Virus (ZIKV) a Public Health Emergency of International Concern due to the virus' emergence in multiple countries globally and the possible association of ZIKV with microcephaly and neurological disorders. There is a clear need to identify risk factors associated with ZIKV infection and microcephaly in order to target surveillance, testing and intervention efforts. Using data collected by surveillance systems, we modeled the correlation between transmission intensity of dengue and chikungunya, with reported microcephaly incidence in Brazilian states and ZIKV incidence in Colombia, as very few microcephaly cases have been reported up to now. We show that there is a strong correlation between the incidence of ZIKV in Colombian departments and the force of infection (but not the crude incidence) of dengue ($R2 = 0.41$, $p<0.001$). Furthermore, we show that there is also a strong correlation between the incidence of microcephaly in Brazilian states and the force of infection of dengue ($R2 = 0.48$, $p<0.001$). Because dengue and ZIKV are transmitted by the same vector, these associations provide further support to the supposition that ZIKV infection during pregnancy causes microcephaly, and raise questions about potential interactions between these two flaviviruses. In addition, they provide an opportunity to project the expected incidence of microcephaly in multiple dengue endemic locations across Colombia and the American continent. If the relationship between dengue FOI and microcephaly incidence seen in Brazil holds in Colombia, we should expect to see 387 cases (95%CI 166-621) of ZIKV associated microcephaly to be reported over the next 7 months. These results are being updated as the ZIKV epidemic progresses and will be expanded to include other countries in the

American continents where data on dengue and ZIKV is available. Detailed knowledge of dengue transmission should be used to target surveillance, testing and intervention efforts against ZIKV and other flaviviruses.

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CORRELATION BETWEEN SABIN POLIOVIRUS SHEDDING AND SUBSEQUENT SEROCONVERSION IN INDIAN CHILDREN VACCINATED WITH MONOVALENT TYPE 3 ORAL POLIOVIRUS VACCINE (MOPV3)

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OPV (oral poliovirus vaccine) shedding in stool after vaccination indicates vaccine virus replication in the gut which results in an optimal immune response to OPV, as evidenced by few studies. The objective of the study was to evaluate the correlation between mOPV3 shedding on day 7 after vaccination and subsequent seroconversion in Indian children aged 6-11 months. A total of 300 infants aged 6-11 months who were seronegative to type 3 poliovirus (neutralizing antibody titre <8) and recruited from Vellore, India as part of a clinical trial evaluating the effect of azithromycin on the immunogenicity of mOPV3 given to healthy infants aged 6-11 months, were included in the study (CTRI/2014/05/004588). Serum samples were collected after 21 days of vaccination with mOPV3 to evaluate seroconversion (antibody titre ≥8). Neutralization test was performed on the serum samples according to the WHO protocol to determine antibody titres against serotype 3 poliovirus. Stool samples were collected 7 days after vaccination to determine Sabin 3 shedding using quantitative real-time polymerase chain reaction (PCR). A Ct value of 40 was used as a cut-off value for positive samples. 160 infants (53.3%) were found to shed Sabin 3 poliovirus on day 7 after vaccination while 140 infants (47.7%) did not shed. Of the 160 infants who shed vaccine virus, 136 infants (85%) seroconverted while 24 infants (15%) did not seroconvert. Of the 140 infants who did not shed vaccine virus, only 14 (10%) seroconverted in contrast to 126 infants (90%) who remained seronegative. Shedding of Sabin poliovirus and seroconversion were strongly correlated (Fisher's <0.001). To conclude, our study found a significant association between mOPV3 shedding on day 7 and subsequent seroconversion (after 21 days of vaccination) in Indian children.

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CHIKUNGUNYA, ZIKA, AND DENGUE IN CALI, COLOMBIA: PRELIMINARY RESULTS OF EPIDEMIOLOGICAL AND GEOSPATIAL ANALYSES

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Vector born disease control is expensive, time consuming, and difficult to achieve. Here, we propose a new method of describing environmental risks in order to more efficiently control *Aedes* born disease in Santiago de Cali, Colombia, an area which is endemic for dengue and chikungunya and currently controlling an outbreak of zika. Geospatial video narratives are a new method of capturing street level data for environmental risks with expert and community based opinion. These data, consisting of local expert interviews and video, are coded and analyzed in a laboratory and compared across space and time as GIS map-layers. Epidemiological analyses are being conducted including geospatial components (geographically weighted regressions, kernel density analysis, hotspot analysis) in addition to traditional incidence data measures. Preliminary dengue and chikungunya data from October 2014 - April, 2016, obtained from the local ministry of health database, SIVIGILA, suggest two primary hotspots (areas with higher than expected case data) which are related to local environmental risks (open sewers, homeless population, lack of trash and sanitation services). Local partners are being engaged to provide expert opinion and guide the work in the neighborhoods most in need. We expect to present the final results of the analysis to community partners and the secretary of health of Santiago de Cali as they continue to control these neglected tropical diseases affecting the most vulnerable populations of the city. New data is currently being cleaned and analyzed and final results are expected by July, 2016.

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A TRIAL TO ASSESS THE THERMOTOLERANCE OF AN INACTIVATED RABIES VACCINE

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Mass dog vaccination is the most efficient way to eliminate human rabies. This study provides the first robust data that the immunogenicity (a surrogate of protection) of an inactivated rabies vaccine (Nobivac® Rabies), stored at temperatures in excess of recommended conditions, is not inferior to that of the vaccine stored in cold-chain conditions. A non-inferiority study was carried out comparing the serological response at 4 weeks post vaccination in dogs inoculated with vaccine stored at elevated temperatures for different durations, with dogs vaccinated with the product stored according to label recommendations. The results showed that the effectiveness of the vaccine at stimulating antibody was not inferior to cold-chain stored vaccine when it was stored for up to 6 months at 25°C or for 3 months at 30°C. Despite being unlikely to result in changes to the authorized storage conditions of this product, the development of thermotolerant vaccines will increase delivery options. For example vaccines could be stored in remote communities with no electricity, thus allowing dogs to be vaccinated throughout the year rather than annually when campaigns pass through. As such, puppies born after a campaign could be vaccinated in a timely manner, reducing the rate at which the inter-campaign vaccination coverage decreases. This will be useful where the 70% coverage target, required for local elimination of the virus from the canine reservoir host, has not been reached. Thermotolerant vaccines stored in remote areas will also provide a human life-saving resource in emergency outbreak situations where rapid vaccination of the dog population is required to control the epidemic. We have not confirmed a 3-yr duration of immunity for the high temperature stored vaccine, however annual re-vaccination is usually practiced for all dogs presented during vaccination campaigns in Africa and Asia. As such this should not be a cause for concern. Given the recent tripartite (WHO, OIE, FAO) commitment to eliminating canine-mediated human rabies by 2030, these results are extremely timely.

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RISK FACTORS FOR ANTIBODY LOSS AFTER HEPATITIS E VIRUS NATURAL INFECTION OR VACCINATION: RESULTS OF A MULTI-SITE COHORT STUDY

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Hepatitis E virus (HEV) is a vaccine-preventable emerging infection causing 20 million infections in developing countries per year. In South East

Asia HEV causes yearly outbreaks, with the majority of disease seen in adults. This is unexpected for an enteric pathogen, suggesting that HEV antibody persistence after exposure is not long-lasting. We revisited 170 subjects with a documented HEV infection from Bangladesh and China and 97 subjects vaccinated with the HEV239 vaccine during a phase III trial in China to retest their serum for anti-HEV antibodies 6 to 10 years after exposure. Overall, 22.1% (95%CI: 17.3-27.6%) no longer had detectable antibodies at follow-up. Antibody loss was greater among the naturally infected subjects compared to the vaccinated subjects, 24.1% (95%CI: 17.9-31.3%) versus 18.6% (95%CI: 11.4-27.7%), although not statistically significant (p=0.292). Among all the subjects, age at exposure was associated with antibody loss, with younger age increasing the risk of antibody loss (RR: 0.87 per 10 years, 95% CI: 0.76-1.00, p=0.057). Among the subjects from Bangladesh, each 10 year increase in age at infection decreased the risk of antibody loss by 50% across univariate and multivariate Poisson regression models (p<0.05). This age-dependent antibody loss could partially explain the wide body of cross-sectional seroprevalence data from SE Asia where a paucity of pediatric infections has been observed. In multivariate models, factors that increased the risk of HEV were generally associated with antibody persistence among the naturally infected subjects, suggesting repeated exposures over time contribute to antibody persistence. This pattern was not found in the vaccinated subjects. This is the first study to compare long term antibody persistence after HEV exposure in naturally infected and vaccinated individuals, exploring host characteristics. The development of a successful, subunit vaccine has increased the need to understand the duration of antibodies and protection after HEV infection and vaccination in order to implement the most cost effective disease control and vaccination strategies.

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ANTIBODIES TO EBOLA IN INTERNATIONAL RESPONDERS TO THE WEST AFRICA EBOLA EPIDEMIC

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The 2014/5 Ebola (EVD) epidemic in West Africa (WA) resulted in a large international humanitarian and research response. Health care workers (HCWs) from WA were disproportionately affected, and a few international HCWs were infected. Since asymptomatic and pauci-symptomatic EVD has been described, we tested international returnees for EVD antibodies. An online consent and survey link were distributed using a snowball technique. Eligibility criteria included never having tested positive for Ebola virus, and not ever having received a filovirus vaccine. Oral fluid collection devices were posted to participants and returned using standard mail. Samples will be analysed using an IgG capture ELISA. Non-exposed controls in the UK will also be tested. Data are stored on a secure database, and analysed using STATA 14. A total of 270 individuals who travelled to WA during the 2014/5 Ebola epidemic completed an online survey. Of these, 155 (57.4%) were women. The majority (253, 93.7%) travelled to Sierra Leone; 13 (4.8%) to Liberia and 14 (1.5%) to Guinea. Roles included, but were not limited to; laboratory (95, 35.2%), clinical (71, 26.3%), epidemiologist/research (23, 8.5%), community engagement/burial (14, 5.2%) and water/sanitation/engineer (4, 1.5%). A total of 139 (51.5%) returnees spent time in the red zone, of whom 21 (7.8%) described direct physical contact with suspected/confirmed EVD patients or their children outside of the red zone, and 99 (36.7%), direct physical contact with survivors. A total of 56 (20.7%) experienced a febrile illness in WA, or within a month of return. Twenty-five (9.3%) had a negative PCR test for Ebola. Of 236 individuals who wore personal protective equipment (PPE), 22 (9.3%) had concerns regarding Ebola exposure during removal. Serological results will be available. A high proportion of international responders reported potential exposure outside of PPE, concerns about exposure when removing PPE, and reported a febrile illness during the incubation period for Ebola. Improvements in training and procedures, and consistency in PPE equipment and removal may mitigate some of this perceived risk.

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MEN'S HEALTH SCREENING PROGRAM: EBOLA VIRUS DISEASE (EVD) SURVIVOR SEMEN TESTING PRELIMINARY FINDINGS — LIBERIA, 2015-2016

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Ebola virus (EBOV) RNA has been detected in semen of EVD survivors > 9 months after disease onset. However, information regarding duration and risk factors for sustained viral persistence in semen is limited. Liberia's Men's Health Screening Program, created to prevent EBOV sexual transmission, offers counseling and semen testing for EBOV RNA by RT-PCR. Males ≥15 years of age with proof of EVD survivorship (e.g., discharge certificate from an Ebola Treatment Unit) were enrolled in a study launched in July 2015. Participants are provided semen testing for EBOV RNA by RT-PCR; counseling on safe sexual practices; condoms with instructions on use; and referrals for healthcare services as needed or requested. In accordance with WHO guidelines, participants are eligible to graduate from the program after receiving two consecutive RT-PCR negative semen tests; with samples collected at least 2 weeks apart. As of February 2016, RT-PCR results were available for 307 participants. In total, 35 (11%) participants had at least one RT-PCR positive semen test. Of these, 21 (7%) participants were ≥ 12 months from ETU discharge at the time the RT-PCR positive semen sample was collected. The longest time after ETU discharge to collection of a semen sample that tested positive for EBOV RNA was 523 days. The median age of participants who had a RT-PCR positive test at program enrollment was significantly older than those who never had a RT-PCR positive test (p=.0031). Excluding participants who enrolled within 90 days from ETU discharge, men aged ≥35 years were more likely to have at least one semen sample test RT-PCR positive compared to men aged <35 years (p=.0024). Frequency of sexual intercourse was not associated with age (p=0.2208). We found persistence of EBOV RNA in semen up to 523 days from ETU discharge, far exceeding previous reports. Sustained viral RNA persistence in semen appears to be associated with older age, an association not previously reported. As frequency of sexual intercourse was not associated with age, differences in viral RNA persistence may be related to other age-related factors such as changes in semen composition or immune function.

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HEPATITIS E INFECTION IN ARGENTINA, FROM IMMUNOCOMPETENT TO IMMUNOCOMPROMISED

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Hepatitis E virus (HEV) is an RNA virus that can cause hepatitis in an epidemic fashion. In immunocompetent individuals, infection with HEV

usually leads to silent seroconversion or to acute self-limited disease. In immunosuppressed individuals, HEV can develop into a chronic infection. Information about prevalence of HEV in immunocopromised subjects outside of Europe or North America is scarce. In this study we addressed the seroprevalence of HEV in immunocompetent and immunocompromised subjects in Argentina and associated risk factors. We performed third generation enzyme immunoassay for determination of IgG and IgM specific antibodies against HEV in 204 subjects infected with HIV, 81 subjects on hemodialysis (HD) and 58 solid-organ transplant recipients. HEV PCR was performed in all samples. Subjects on HD and transplant recipients were evaluated regarding social habits and potential risk factors. Results were compared to 433 HIV-negative, immunocompetent controls from our center. In our entire HIV-positive group we found 15 of 204 samples to be positive for HEV IgG (7.3%), compared to 19 of 433 samples (4.4%) in the control group. Interestingly, we found significantly lower CD4 counts on HEV-positive samples compared to HEV-negative samples (average CD4 count of 234 vs 422 mm^3, p=0.01) indicating that patients with lower CD4 counts were more likely to be HEV IgG positive. Eight out of 81 subjects (9.8%) on HD and 5 of 58 (8.6%) of transplant recipients were positive for HEV IgG. Half of HEV seropositive patients in the HD group had positive IgM for HEV. There was no association between HEV serostatus and consumption of pork, alcohol, potable water or history of blood transfusion. There was a weak, but significant, association between fish consumption and HEV positivity. Only 1 sample showed a positive PCR for HEV, within the HIV group. In conclusion, our study found an increased seroprevalence of HEV IgG in subjects infected with HIV, on HD and solid-organ transplant recipients in Argentina. However, the only significant difference compared to controls was on HIV-infected patients with low CD4 counts.

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CHARACTERISTICS AND OUTCOMES OF PEDIATRIC PATIENTS WITH EBOLA VIRUS DISEASE (EVD) ADMITTED TO TREATMENT UNITS IN LIBERIA AND SIERRA LEONE: A RETROSPECTIVE COHORT STUDY

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This retrospective cohort study describes the clinical characteristics and outcomes of children <18 years with PCR-confirmed EVD among patients evaluated for suspected EVD in West Africa. Demographic, epidemiological and clinical data were collected systematically from patients with documented EVD admitted to five Ebola Treatment Units (ETU) in Liberia and Sierra Leone during 2014/2015. Standardized care was provided to all patients based on International Medical Corps protocols. The pediatric cohort was described in aggregate and stratified by age group. We analyzed associations between mortality and patient characteristics. Among the 128 pediatric cases admitted, 122 had PCR-confirmed EVD. Incidence in females (56%) and males (44%) was similar. The overall mortality rate was 57%. Stratified by age, mortality was 89% for <5 years, 43% for 5-9 years, 41% for 10-14 years, and 25% for 14-17 years (p<0.001). Mortality for children aged <5 years was significantly higher than children 5-17 years old (89% vs 38%, p<0.001). At triage, prominent features included fever (79%), anorexia (68%), and weakness (64%). Throughout the duration of illness, the most frequent features were weakness (92%), fever (85%), anorexia and diarrhea (both 80%). Of those presenting without fever (n=26), 73% developed fever after triage. Of those patients without fever throughout hospitalization, 3 of 6 (50%) died. Hemorrhagic features were present in 5% at triage and 45% anytime during admission. The mortality of patients that developed bleeding at any time during admission was significantly higher (58% vs 27%, p=0.002). The median length of stay was 9 days (range 1-31 days). Length of stay was significantly higher for those that survived compared to those that died (16 vs 6 days, p=0.001). In summary, pediatric EVD patients aged <5 years had significantly higher mortality. One out of every five children presented without fever. Prominent features of EVD in children included constitutional and gastrointestinal signs and symptoms. Hemorrhagic features developed in less than half of pediatric patients, but were associated with significantly higher mortality.

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SALIVA FOR DETECTION OF ANTIBODIES FOR MEASLES, MUMPS AND RUBELLA TO CONFIRM VACCINE STATUS IN TEENAGERS

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Vaccines had resulted in the fall of prevalence of several childhood viral infections in the past decades, also affecting boosting exposure to environmental viruses. Previous concepts of lifelong immunity induced by live virus vaccine have been abandoned due to several outbreaks occurring in vaccinated young adults, obliging revaccination specially for preventing congenital rubella. Serology has been considered the main approach to measure individual protection but demands low adherence blood collection. Saliva could be an alternative for the detection of *Toxoplasma gondii* specific antibodies, due to IgG exuded from crevicular liquid. Saliva collection is noninvasive, simple and inexpensive, with high adherence for children and other protected groups. In this work, we show the development of ELISA for detection of specific IgG against measles, mumps and rubella virus, using a protein A IgG capture and biotinylated recombinant antigens as probes. Anti T.gondii IgG detection was used as control. Samples were collected in public high schools, during an exposition of transmissible diseases, with voluntary collection after parental approval. Vaccination was identified by individual vaccine files. Negative samples were obtained from discarded sera from Pediatric Center Lab, from children below vaccine age. All samples were tested on 384 wells plates, adsorbed with Protein A (10mg/ml), reacted with saliva IgG 10 x concentrated by clearing and ethanol precipitation. Commercial recombinant antigens from measles, mumps, rubella and *T. gondii* extracts were biotinylated and allowed to react to bound IgG, followed by avidin peroxidase and TMB reaction. All the assays were efficient for distinction of reactive sera, without false negative or false positive results as compared to unvaccinated young children sera. These tests could be easily transformed in high throughput assays, allowing the determination of individual vaccine status with consequent preventive measure as revaccination. Saliva IgG will enable vaccine control without the need for blood collection.

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PRESENTING SYMPTOMS AND CHARACTERISTICS OF PATIENTS WITH EBOLA AND MALARIA ADMITTED TO EBOLA TREATMENT CENTERS IN SIERRA LEONE

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Discriminating between Ebola Virus Disease (EVD) and malaria is difficult when clinical testing is not immediately available. This retrospective study describes characteristics of patients admitted to Ebola Treatment Units (ETU) in Sierra Leone during the recent epidemic in West Africa with variable states of EVD and malarial infections. Data was collected from three International Medical Corps ETUs in which all patients received standardized care including Artesunate combination treatment (ACT). The population was stratified by infection status as malaria negative/EVD negative (m-/e-), malaria positive/EVD negative (m+/e-), malaria negative/EVD positive (m-/e+) and malaria positive/EVD positive (m+/e+). These groups were analyzed for inter-group variation in characteristics, symptomatology and outcomes. Among 1548 admitted patients, 753 patients had diagnostic test results available for EVD and malaria. There were 431 (57.2%) m-/e-, 180 (23.9%) m+/e-, 108 (14.4%) m-/e+,

and 34 (4.5%) m+/e+ analyzed in the cohort. Patients diagnosed with malaria had significantly younger median ages at 23 and 20 years for the m+/e- and m+/e+ respectively versus 32 years among both the m-/e- and m-/e- groups (p<0.001). Females accounted for a larger proportion of EVD cases than males (42% m-/e-, 42% m+/e-, 65% m-/e+ and 68% m+/e+; p<0.001). Patients were significantly more likely to have abdominal pain if they had malaria rather than EVD (49% m+/e-, 36% m-/e+; p=0.043). Presence of anorexia, diarrhea, bone pain, vomiting, cephalgia, dyspnea and abnormal bleeding were not significantly different between m-/e+ and m+/e- groups. Distinguishing between EBV and malaria before rapid diagnostic testing is available presents a difficult diagnostic challenge. Significant overlap exists in the clinical presentation of patients with variable EVD and malarial infection statuses. This may justify the continued use of ACT in an EBV epidemic. Better understanding of such characteristics among variably infected patients may enhance development of response protocols and care in future EVD epidemics.

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CHARACTERIZATION OF SEVERE FEVER WITH *THROMBOCYTOPENIA* SYNDROME VIRUSES (SFTSV) FROM PATIENTS IN KOREA, 2015

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Introduction Severe fever with thrombocytopenia syndrome (SFTS) is emerging infectious disease characterized by acute febrile, thrombocytopenia and gastrointestinal symptoms. It is caused by SFTS virus (SFTSV), in the genus of Phlebovirus (family Bunyaviridae). Since the first reported case of SFTS in South Korea in 2013, we collected serum samples from hospitalized patients who experienced symptoms of SFTS. The major clinical symptoms and laboratory parameters of SFTS are fever, thrombocytopenia, leukopenia, and elevated serum hepatic enzymes, and SFTS patients usually die due to multiple organ failure. SFTSV was presumably transmitted by ticks, because it has been detected in Haemaphysalis longicornis ticks. Methods and Materials Total RNA extracted from serum was amplified with one-step reverse-transcriptase polymerase chain reaction (RT-PCR), designed to detect a portion of the viral N and Gc protein gene using specific primers for S or M segment. After analyzing aligned nucleotide sequences, we constructed the phylogenic tree based on partial S or M segment sequences. We tried to isolate viruses from patient by infection VeroE6 cells with the sera. Results We conducted RT-PCR with total RNA which is extracted from the patient sera. Among the 833 samples, seventy-nine samples are resulted in positive. The nucleotide sequences were assembled by the SeqMan program implemented in DNASTAR software (version 5.06; Madison, WI, USA) to determine the consensus sequences. Nucleotide sequence of the Korean strains showed 93 to 98 % homology to Chinese and Japanese strains. We also isolated 45 SFTSVs among the virus-detected 79 samples. Conclusion We examined the clinical specimen from the suspected case of SFTS in Korea. We detected 79 SFTSVs of 833 patient sera by RT-PCR, and isolated 45 viruses among them. Nucleotide sequences of positive samples were not only included in SFTSV by the phylogenic analysis but also formed the Korean strain group.

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EFFICACY AND SAFETY OF A LOW-COST, HEAT-STABLE ORAL ROTAVIRUS VACCINE AGAINST SEVERE ROTAVIRUS GASTROENTERITIS IN NIGER

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Prevention of rotavirus disease through vaccination is a public health priority, and in 2009, the World Health Organization recommended rotavirus vaccination be introduced in all countries to reduce disease burden and mortality among young children. Two live oral attenuated rotavirus vaccines are globally licensed and WHO prequalified for the prevention of rotavirus gastroenteritis. Safety and efficacy of these vaccines has been established in high- and middle-income countries, but vaccination in sub-Saharan Africa, where there is the largest burden of rotavirus-related mortality, presents certain logistical and financial challenges. BRV-PV is a low-cost and heat-stable rotavirus vaccine manufactured by the Serum Institute of India, Limited whose introduction may help minimize the burden on already-strained national immunization programs throughout sub-Saharan Africa. We conducted a double-blind, placebo-controlled randomized phase III event-driven trial in Niger to assess the efficacy and safety of BRV-PV against severe rotavirus gastroenteritis in infants in Niger. Infants were randomized in a 1:1 ratio to receive three doses of BRV-PRV or placebo at approximately 6, 10, and 14 weeks of age. Facility and home-based surveillance is being conducted from 28 days post Dose 3 (gastroenteritis) and from the moment the first dose (serious adverse events) until 2 years of age. As an event-driven trial, the primary efficacy analysis was planned when 117 cases of severe rotavirus gastroenteritis are confirmed. Vaccine efficacy against severe rotavirus gastroenteritis and risk of serious adverse events, including hospitalization, intussusception and death will be presented. Evidence supporting the efficacy and safety of BRV-PV vaccine in an African setting would support the pre-qualification of and increased access to rotavirus vaccine across Africa.

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CROSS-REACTIVE ANTIBODIES INFLUENCE IMMUNOGENICITY OF LIVE ATTENUATED VACCINES

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Epidemic viral diseases have become increasingly prevalent, as evidenced by the spread of ebola and zika. Among the different anti-viral strategies, vaccination, particularly with live attenuated vaccines (LAV), has seen exceptional success. However, due to previous exposure to pathogens and vaccines during the lifetime of an individual, many would have pre-existing cross-reactive antibodies that can potentially affect efficacy of live-attenuated vaccines (LAVs). To understand how cross-reactive antibodies affect LAVs, we designed a randomized trial design where subjects were subjected to Japanese encephalitis (JE) inactivated vaccine followed by the LAV yellow fever (YF) vaccine. We observed that YF vaccine immunogenicity is affected by the levels of cross-reactive JE antibodies. Transcriptional profiling of the patients highlighted that semaphorins and T-cell related cytokines were significantly correlated with YF immunogenicity. Further laboratory studies reveal that semaphorins that are expressed in Fc-receptor bearing antigen-presenting cells were directly upregulated by activating Fc-receptor signaling. That semaphorins have crucial roles in antigen presentation and T-cell responses suggest that cross-reactive antibodies can be exploited to improve LAV efficacy.

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VERTICAL TRANSMISSION OF CYTOMEGALOVIRUS IN A RURAL MOZAMBICAN HOSPITAL

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Congenital cytomegalovirus (CMV) infection is the most prevalent congenital infection worldwide, varying the prevalence from 0.2% to 2%. This prevalence is higher in developing countries and among HIV infected newborns although it is decreasing among exposed but not infected

infants, following the increasing use of antiretroviral therapy for prevention of mother-to-child transmission (MTCT). We aimed to assess CMV vertical transmission prevalence and risk factors associated among newborns born in a rural Mozambican maternity. A cross sectional study was conducted on pregnant mothers attending Manhiça District Hospital at delivery. Blood cord samples in filter paper and placenta biopsy were collected for CMV detection measure by PCR. CMV seroprevalence and HIV status were also investigated in recruited pregnant women. One hundred and twenty mothers were recruited at delivery, mean age was 25,1 (±7,6) years and mean of gestational age at recruitment was 38,8 (±0,6) weeks. Prevalence of HIV infection among them was 27.5% (33/120) and only 28.3% were taking antiretroviral to prevent MTCT. One hundred and twenty three pregnancy outcomes were delivered. 5.8% (7/120) were premature, 1.7% (2/120) were stillbirths, 2.5% (3/120) born with malformations (2 with polydactyl and one with spine bifida) and 12.5% (15/20) had low birth weight. Data of three newborns were missing. CMV PCR was positive in 3 of 116 cord samples collected (2.6%) and only one child was exposed but not infected to HIV. 100% of them born asymptomatic at birth and at 6 months follow-up. Risk factors associated to vertical transmission of CMV were not found. We will present results of placental biopsies and maternal seroprevalence. Our results showed a higher prevalence of congenital CMV than studies in developed countries but lower than reports from low-income countries. Although it is an important cause of hearing loss completely neglected, it would be premature to consider newborn CMV screening in resource-poor settings because the disease burden from congenital CMV and the cost/benefit ratio of long term follow-up have not been defined.

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IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF MATING-DELIVERED MALE SEMINAL FLUID PROTEINS IN THE MALARIA VECTOR *AEDES*

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Anopheles gambiae mosquitoes are one of the primary vectors of human malaria, which represents a major public health burden globally. Reproductive success in these mosquitoes relies on a single mating, therefore characterizing their reproductive biology could offer a promising opportunity to interfere with their life cycle. *An. gambiae* male mosquitoes produce a complex cocktail of seminal fluids in their accessory glands (MAGs), including proteins (Acps), and lipids that are packed in a gelatinous mating plug and transferred to females during copulation. Female receipt of this plug induces large physiological and behavioral changes including refractoriness to further insemination, egg laying and activation of sperm storage mechanisms. We recently demonstrated that the steroid hormone 20-hydroxyecdysone (20E) in part mediates these change in females. However the identity and function of seminal fluid proteins in regulating the female post mating response remains largely unknown. We employed an *in vivo* stable isotope labeling technique coupled to mass spectrometry to identify male proteins transferred to females during mating. Female mosquitoes were labeled via feeding yeast containing stable isotope ^{15}N, which masked the female proteome, allowing identification of male specific proteins. First, the proteomic composition of unlabeled male reproductive tissues (MAGs and testes) was determined, which facilitated the spatio-temporal localization of male proteins within 5 female tissues (atrium, spermatheca, ovaries, hemolymph and head) at three time points after mating (3h, 12h and 24h). Notably we detected a total of 180 unique male proteins transferred to females, including 45 novel Acps. Male proteins transferred to the spermatheca maybe essential in coordinating sperm viability, and strikingly a MAG specific protein of unknown function was detected at all time points after mating in this tissue, suggestive of a stable, long term association with sperm. Ongoing functional knock-out analysis of this and other candidates are revealing the fundamental role of male transferred proteins in female reproductive success.

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FBN30 IS A PATHOGEN RECOGNITION RECEPTOR AGAINST *PLASMODIUM* INFECTION IN *ANOPHELES GAMBIAE*

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Malaria is a worldwide health problem that affects two thirds of the world population. Invasion of *Plasmodium* through anopheline mosquitoes is an obligatory step for malaria transmission. Through genome-wide association studies, we identified significant association between genetic variations in FBN30 and the *P. falciparum* parasite infection in *Anopheles gambiae* mosquito populations from malaria endemic areas in Kenya. FBN30 sequence analysis shows that the fibrinogen-like domain (FBG) at the C-terminal is conserved across a range of mosquito species. In this study, we hypothesize that FBN30 works as a pathogen recognition receptor (PRR) in the defense against *Plasmodium* infection. Firstly, we expressed FBN30 in insect High Five cells and studied its biochemical features. The results show that insect cell expressed FBN30 is a secreted protein which can form a dimer through disulfide bond between two subunits which indicate four disulfide-bond linked homodimers to form a tetramer by non-covalent bond. In addition, we determined the cysteines, which are involved in the intra-chain and inter-chain disulfide bridge respectively. Secondly, we evaluated the expression efficiency of two FBN30 variants (FBN30(C/C) and FBN30 (T/T)) in mosquito cells, Moss55 and Sau5B. The results support that wild type mosquitoes in Kenya, with the genotype of FBN30(C/C), were more susceptible to *Plasmodium* infection than those with the genotype of FBN30 (T/T). The susceptibility is attributed to a lesser expression of FBN30 observed in mosquitoes with the non-synonymous mutation, which results in Phe10Leu in the signal peptide. Also we determined that FBN30 in *An. gambiae* only exists in hemolymph. Finally, ELISA and indirect immunofluorescence assay proved that FBN30 proteins bind to asexual and sexual stage of both *P. berghei* and wild type of *P. falciparum*. Based on all these data, we concluded that FBN30 is a PRR molecule in the mosquito innate immune system, which is critical for *An. gambiae* defense against *Plasmodium* infection.

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IDENTIFICATION OF GLYCOSAMINOGLYCANS IN *ANOPHELES NEIVAI* AND *A. ALBIMANUS AND* ITS ROLE IN MALARIA TRANSMISSION

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Despite the advances in research related to malaria it is accepted and clear that interventions to limit transmission by the vector are not enough, therefore the search for new strategies for malaria control is necessary. The sexual cycle of *Plasmodium* or sporogonic cycle occurs in mosquitoes of the genus *Anopheles*. It has been reported that malaria transmission is due to glycosaminoglycans (GAGs) which are present in the epithelium of the midgut and salivary glands of the mosquito and its interactions with lectins in the parasite, it is thought that the presence of such molecules is necessary to promote maturation from ookinete to oocysts. Although some GAGs such as chondroitin sulfate and heparan sulfate have been identified in experimental models of interaction between host-parasite,

the real participation of those GAGs in malaria transmission is unknown. Therefore *An. albimanus* and *An. neivai* from the department of Chocó-Colombia has been taken as a natural model with the purpose of identify the GAGs present in its tissues and to establish its relationship in the transmission of malaria. Adult mosquitoes were collected and larvarian forms were cultured in the laboratory. All adult forms were used for identification by classical taxonomy and confirmed by BarCode technique. The midgut tissue of two sets of malaria vectors were obtained and were used for GAGs analysis by mass spectrometry. We will discuss about some subtle differences in the GAGs profiles between different species of *Anopheles*. This fact could explain the ability to permit or inhibit the *Plasmodium* parasites maturation and transmission by vectors. This knowledge will help to find out new strategies for blocking the transmission cycle.

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DISCOVERING THE BINDING PARTNER(S) OF MOSQUITO MIDGUT FREP1 IN *PLASMODIUM* PARASITES

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We previously identified a mosquito midgut protein FREP1 in peritrophic matrix that can facilitate *Plasmodium* infection in *Anopheles gambiae* mosquitoes through binding to gametocytes and ookinetes. We proposed a FREP1-mediated parasites invasion model. This project aims to identify the parasite-expressed FREP1-binding partners (FBPs). Incubating insect cell-expressed recombinant FREP1 with *P. berghei* infected cell lysates pulled down several specific bands by anti-FREP1 antibodies. One of the bands was identified by mass spectrometry to be a 27 kDa, PEXEL motif-containing protein. This protein also has a trans-membrane domain. Insect cell-expressed this candidate protein confirmed its interaction with FREP1. The FBPs discovered in this report will improve our understanding of the molecular mechanism of FREP1-mediated *Plasmodium* invasion pathway, which can be targeted by novel approaches for malaria control.

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NEW USES FOR AN OLD TECHNOLOGY TO CONTROL ZIKA VECTORS IN URBAN TROPICAL ENVIRONMENTS

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The recent epidemic of Zika virus transmission in the Southern Hemisphere has brought heightened awareness for the control of its vector *Aedes aegypti*. Dumping, removing or treating every water-holding container, especially in large urban neighborhoods, maybe impractical. The dense clouds of insecticide produced by the "old" thermal fog technology may now be advantageous for penetrating cryptic habitats. Compatible aqueous mosquito adulticide formulations for use in thermal foggers are commercially available that replace the dense fog with a fine mist. Therefore, we speculated "Could currently available aqueous larvicide formulations be applied by a thermal fogger and remain efficacious in cryptic larval habitats for the control of Zika vectors?" We initiated semi-field studies to determine penetration distance and associated *Ae. aegypti* larval mortality in a forest canopy (cryptic) environment using *Bacillus thuringiensis* var *israelensis* (*Bti*) (Vectobac WDG, AI 37.5%). Vectobac was applied at the maximum label rate with a hand held IGEBA TF 34 thermal fogger (fitted with water conversion kit) to empty 0.5-L clear plastic containers placed at 7, 14, 21, and 28m from application source at Camp Blanding, FL. Treated containers were returned to the laboratory where dechlorinated water and late second to early third instar *Ae. aegypti* were added to each container. The greatest amount of mortality (91-100%) occurred at 7m. We then operationally applied the *Bti* product, at maximum label rate, to a 0.2 ha urban tropical urban environment located in Key West, FL using the same larval evaluation bioassays above. Containers were placed randomly in cryptic areas within the area. Vectobac provided 99.9% larval mortality at 24h and 100% at 48h. Operationally, we found that thermal fog technology can be an effective tool for control of larval *Ae. aegypti* in cryptic tropical urban environments.

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INFLUENCE OF BLOOD MEAL ON SUSCEPTIBILITY TO PYRETHROIDS IN *ANOPHELES GAMBIAE* FROM WESTERN KENYA

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Vector control is based on the use of insecticides. To ensure sustainable vector control we need to understand the other factors driving insecticide resistance and consequently threatening the sustainability of malaria vector control programs targeting the indoor resting mosquitoes. The aim of this study was to determine the influence of blood meal status on pyrethroid tolerance in field collected population of *Anopheles gambiae* from western Kenya. Field-collected mosquito larvae were reared to adulthood alongside the laboratory susceptible reference Kisumu strain. Female adults from the two populations were monitored for deltamethrin resistance using WHO bioassays at different gonotrophic stages. Metabolic assays were then performed to assay the level of detoxification enzymes. The WHO bioassay results showed increased resistance on younger female's (2-5 days old) field collected population with different gonotrophic status (mortality ranged from 36-83%). Older females (14-15 days) from the same population with different gonotrophic status showed reduced resistance to the same insecticide (85-98%). Biochemical estimations on younger females (2-5 days old) revealed significantly ($P<0.05$) higher levels of oxidase, non-specific esterase and glutathione-s-transferases activity in the blood fed and half gravid survivors *of An. gambiae* as compared to unfed survived individuals. For older females (14-15 days) of the same population, blood fed and half gravid survivors showed significantly higher oxidase and glutathione-s-transferases activity as compare to unfed and gravid survivors. Kisumu susceptible population showed 100% susceptibility with no significant elevation in enzyme activities following a blood meal. These results indicate that blood feeding status plays an important role in the toxicity of deltamethrin due to some physiological changes following feeding that confers increased tolerance to mosquitos.

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NEW MATRIX-RELEASE FORMULATION, SUMILARV®2MR CONTAINING PYRIPROXYFEN FOR LONG LASTING CONTROL OF *AEDES AEGYPTI* LARVAE

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Aedes aegypti is a vector of human viral diseases, such as dengue, Zika, chikungunya, and yellow fever. Indoor and outdoor water storage containers are the main breeding habitats for this species in Southeast Asia, Central and South America, and also in some savannah areas of Africa. A new long lasting "matrix-release" formulation, SumiLarv®2MR,

containing 2% pyriproxyfen has been developed to control container-breeding *Ae. aegypti*. Pyriproxyfen is an insect growth regulator with a very low mammalian toxicity that inhibits the emergence of adult mosquitoes. Pyriproxyfen is recommended for use in drinking water by the World Health Organization (WHO). The key feature of SumiLarv®2MR is the controlled slow release of pyriproxyfen so that an effective concentration of active ingredient is maintained in treated water for at least six months after treatment. A simulated-field evaluation using plastic vessels containing 40 L water showed that the efficacy of SumiLarv®2MR lasted for at least 36 weeks, irrespective of the frequency of water replacement (half or full water replacement every week). A field trial conducted over a year in a rural village in Lao PDR where *Ae. aegypti* breeds throughout the year, with SumiLarv®2MR applied every 6 months to domestic water storage containers, resulted in a significant reduction in larval density. The long-lasting efficacy of SumiLarv®2MR demonstrated in these trials will reduce the number of treatments required per year and will therefore enable significant reductions in operational costs. These results are promising for the future long term control of *Ae. aegypti* and the diseases that this insect transmits. SumiLarv® is a registered trademark of Sumitomo Chemical Company Ltd.

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INHIBITION OF ADULT EMERGENCE OF *AEDES AEGYPTI* USING A CONTROLLED RELEASE FORMULATION OF PYRIPROXYFEN (SUMILARV® 2MR) OVER SIX MONTHS IN CAMBODIA: A CLUSTER RANDOMIZED TRIAL

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Dengue is one of the most rapidly spreading mosquito-borne viral diseases in the world, and without a cure or widely available effective vaccine the best measures to control dengue are through vector control and the avoidance of mosquito bites. Pyriproxyfen (PPF) is a juvenile hormone analogue that interferes with the metamorphosis of juvenile mosquitoes and due to its favorable mammalian toxicity profile is ideal for use in vector control activities. A new slow-release PPF matrix release formulation (Sumilarv® 2MR) was developed and is recommended for six months (considerably longer than alternatives which reduces operational costs). While small scale field studies have been conducted, this is the first large scale field evaluation, where site-specific processes may modify the duration of residual effects relative to controlled experiments. Such large scale field trials therefore measure effectiveness, rather than efficacy, and are of strategic importance to control programs. A cluster randomized, controlled superiority trial was developed to assess the effectiveness of Sumilarv® 2MR. The trial based in Kampong Cham, Cambodia includes 96 sentinel containers from 20 clusters and runs from November 2015 to May 2016. The clusters were randomly assigned to an intervention or control arm. Within randomly selected households the small water jars most commonly used were selected as sentinel containers. Due to the large amount of water replacement the results likely indicate the lowest inhibition values in the household. The primary outcome was the percentage inhibition of emergence in monthly 250 mL water samples. Each lab assay was conducted with 25 lab reared third instar larvae. The beakers were monitored for larvae and pupal mortality and adult emergence once daily for eleven consecutive days or until the last adults emerged or all remaining pupae died. The amount of water replacement in water jars was also recorded to adjust the IE results from sentinel containers. The results can be used by the control programs to assess the long term efficacy and cost effectiveness of Sumilarv® 2MR under operational conditions.

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STUDIES ON KNOCKDOWN RESISTANCE (KDR) MUTATIONS IN *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* IN INDIA

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Knockdown resistance (kdr) is one of the mechanisms of insecticide resistance in insects caused by the reduced target site sensitivity i.e. voltage gated sodium channel (VGSC) rendering it less sensitive to DDT and pyrethroids. We evaluated insecticide susceptibility and its underlying kdr mechanism in eight *Aedes aegypti* and five *Ae. albopictus* field populations from India. Field population were collected from four different geographical regions of India viz. North, South, East and Central covering 18 districts of ten states. Adult bioassays revealed varying levels of resistance to DDT, permethrin and deltamethrin in all *Ae. aegypti* populations and susceptibility to pyrethroids in *Ae. albopictus* populations tested. Molecular screening for common kdr mutations, revealed the presence of five mutations viz. S989P, V1016G, T1520I, F1534C/L. Three PCR based assays; DNA sequencing, ASPCR, PCR-RFLP were used for genotyping of twelve global kdr alleles. Two novel mutations were observed, first at T1520 (ACC) residue where a C>T substitution at the second position of codon results in amino acid change to Isoleucine (ATC). Second mutation was an alternative point mutation at F1534 (TTC) residue where a substitution of T>C at the first position of codon results in an amino acid change to Leucine (CTC). No kdr mutation was observed in any field population of *Ae. albopictus*. ASPCRs were not accurate so three PCR-RFLP assays were developed. Representative samples of all genotypes were sequenced to validate the newly developed PCR based assays for *Ae. aegypti*. DNA sequencing data were in agreement with the genotyping results. Genotyping results showed that 989P is linked to 1016G and novel mutation 1520I was always found with 1534C allele. Present study confirmed the presence of DDT and pyrethroid resistance among *Ae. aegypti* populations in India and for the first time reported kdr mutations in this species from India including two novel mutations. Results of present study lead us to infer that, at least five kdr mutations (S989P, V1016G, T1530I, F1534C and F1534L) can be seen as a potential marker for DDT/pyrethroid resistance.

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EVALUATING THE POTENTIAL OF REUSING LARVAL REARING WATER IN SUPPORT FOR THE STERILE INSECT TECHNIQUE (SIT) OR OTHER MASS PRODUCTION PROGRAM: EFFECT ON DEVELOPMENT AND QUALITY OF *ANOPHELES ARABIENSIS* MOSQUITOES

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The success of a mosquito mass rearing operation for sterile insect technique (SIT) or other release application relies on a reliable supply of water of sufficient quality for optimal larval development. An estimated 250 litres of water is required to raise 200,000 eggs in one larval rearing rack. Yet, many arid and/or seasonally arid countries face the difficulties of acute water shortage, deterioration of water quality, and environmental constraints. The reuse of water to rear successive generations of larvae is attractive as a way to reduce water usage and running costs, and help to make this control method viable. Therefore, we have initiated work at the IPCL to assess whether dirty water is a suitable rearing medium that could replace the clean dechlorinated water that is currently routinely used. Results indicated that reusing dirty water or using a 50:50 mix of clean and dirty water did not affect egg hatching. Moreover, no difference was found in time to pupation, larval mortality or sex ratio when first-

instar larvae were added to clean water, dirty water, or a 75:25, 50:50 or 25:75 mix of clean and dirty water and reared until emergence. When late-instar larvae were put back into their own rearing water, there was no effect on pupation rate, emergence rate or female longevity, though male longevity was reduced. When reared from first-instar larvae, however, dirty water decreased pupation rate, emergence rate, body size, and adult longevity. However no response variable differed significantly between recycled water (reverse osmosis and ultrafiltration) and clean water. This suggests that recycling dirty water necessarily restore overall performance or mosquito quality. Re-used larval-rearing water has no impact on egg hatching, development time or mortality of the immature stages of *Anopheles arabiensis*. However, dirty water is not suitable for the production of high quality adult mosquitoes. Recycling processes improve water quality and increase insect quality. These findings may have important implications for the implementation of the SIT in areas where clean water is a scarce or costly resource.

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DISTRIBUTION AND FREQUENCY OF INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* S.L. POPULATION IN LIBERIA

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Liberia's malaria vector control program relies on insecticide-based interventions and may be compromised by the emergence, intensity, and spread of insecticide resistance among malaria vectors in the country. The distribution and frequency of *Anopheles gambiae* s.l. resistance to five insecticides were examined in six counties from June to December 2015. Three of the six counties included in this survey (Bong, Grand Bassa, Margibi) conducted indoor residual spraying (IRS) from 2009 to 2013, while in the other three counties (Nimba, Gbarpolu, Grand Gedeh), IRS was not done. Non-blood-fed, 3 to 5-day old, female mosquitoes reared from field-collected larvae were exposed to WHO insecticide impregnated and control papers. Four classes of insecticides were tested against *An. gambiae* s.l.: pyrethroid (deltamethrin 0.05% and alpha-cypermethrin 0.05%), carbamate (bendiocarb 0.1%), organophosphate (pirimiphos-methyl 0.1%), and organochlorine (DDT 4%). About 100 female *An. gambiae* s.l. were tested. Mortality was recorded after the 24-hour holding period. *An. gambaie* s.l. populations from all six sites were fully susceptible to pirimiphos-methyl (100% mortality rate). Full susceptibility to bendiocarb was observed only in Grand Gedeh. Probable resistance to bendiocarb was detected in four sites (Bong, Nimba, Margibi, and Grand Bassa), with 97%, 94%, 97%, and 94% mortality rates, respectively, while resistance to bendiocarb was detected in Gbarpolu (89% mortality). Tested mosquitoes were resistant to deltamethrin (22%-73% mortality) and to alpha-cypermethrin (5%-47% mortality) in all sites. The 24-hour mortality for DDT tested in five of the six sites was 2%-49%. Comparison of susceptibility of *An. gambiae* s.l. populations between counties with and without IRS did not show a significant difference ($p > 0.05$). Additional assays are needed over time to map the rest of Liberia and track insecticide resistance as interventions are scaled up.

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IMAGING AND QUANTITATIVE MICROANALYSIS OF PYRETHROID INSECTICIDES ON THE SURFACE AND INTERIOR REGIONS OF LLIN FIBERS USING TIME-OF-FLIGHT SECONDARY ION MASS SPECTROMETRY

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Quantitative chemical mapping of permethrin and deltamethrin on the surface of and within polyethylene long-lasting insecticidal net (LLIN) fibers was carried out using time-of-flight secondary ion mass spectrometry (ToF-SIMS). This method uses a highly focused (0.3μm) Bi3+ primary beam to sputter the fiber surface, and ionic molecular fragments (secondary ions) from the top 1-2 monolayers of the fiber are extracted into a mass analyzer for chemical identification. By rastering the primary beam, a two-dimensional chemical map can be generated for a larger (e.g., 500μm^2) sampling area at submicron resolution. Subsurface regions were exposed for analysis by using a Cs+ beam to remove overlying material, thereby permitting three-dimensional mapping. Calibration standards for quantitative analysis were prepared by implanting known quantities of Cl- and Br- ions in polyethylene films and LLIN fibers. Limits of detection were determined to be 0.051 and 0.0088 weight percent for permethrin and deltamethrin, respectively. Both insecticides were observed as discrete deposits on fiber surfaces in as-received samples. The regeneration process was directly observed in samples that were washed and incubated, and the regenerated insecticide was more diffusely distributed on the surface than in the as-received samples. For permethrin-containing fibers, discrete high-concentration domains of insecticide were found within the fibers suggesting that they are supersaturated with insecticide at ambient temperatures. This method shows great potential for measuring the portion of bioavailable insecticide on LLIN fibers and directly observing the insecticide regeneration dynamics in "insecticide-incorporated" LLINs. It is also potentially applicable to the microanalysis of other active ingredients being considered for use in LLINs, such as piperonyl butoxide (PBO).

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ANOPHELES ALBIMANUS MICROBIOTA AND LINKS TO INSECTICIDE RESISTANCE: A SHOTGUN METAGENOMIC SEQUENCING APPROACH

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Understanding the factors that contribute to insecticide resistance is needed to mitigate its threat to vector control, particularly in Latin America, where vector control is being intensified as part of regional malaria elimination strategies. Next Generation sequencing has been applied to investigate the role of mosquito microbiota in various mosquito behaviors and functions. We report here for the first time the results of shotgun metagenomic DNA sequencing to characterize mosquito microbiota in relation to insecticide resistance phenotypes. Following evidence of a link between insecticide resistance and insecticide detoxifying endosymbionts such as *Burkholderia sp.* in stinkbugs, we hypothesized that the mosquito microbiota may contribute to insecticide resistance. *Anopheles albimanus* from northern Peru were sequenced using the Illumina MiSeq platform. Resulting data were quality checked using PRINSEQ and Trimmomatic quality control tools, and taxonomic composition analysis was performed using GOTTCHA, Kraken and MG-RAST. Similar to other mosquito species, our data showed that *An. albimanus* microbiota was dominated by a single taxon: Proteobacteria at the phylum level, and *Enterobacter* at the genus level. Up to 50 bacterial genera, including *Burkholderia sp.*, constituted the remaining microbiota.

Our ongoing analyses will further identify the core microbiota and their functions in insecticide resistant and susceptible strains of *An. albimanus* from Peru, Guatemala, and Mexico.

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A CLUSTER RANDOMIZED TRIAL TO COMPARE BENDIOCARB AND DELTAMETHRIN FOR INDOOR RESIDUAL SPRAYING ON BIOKO ISLAND, EQUATORIAL GUINEA

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Indoor residual spraying (IRS) - spraying the interior walls of houses with insecticide - has been used on Bioko for malaria control since 2004. The insecticide bendiocarb was used biannually from 2005 until 2012; and during this period prevalence of malaria and under 5 mortality dropped substantially. To reduce costs, biannual IRS with bendiocarb was replaced by an annual round of IRS with a long lasting formulation of deltamethrin in 2013, based on reported susceptibility of local vectors to both insecticides. A marked increase in malaria prevalence in children 2 to 14 years in the same year prompted a cluster randomised trial to be carried out to compare the effectiveness of bendiocarb and deltamethrin for IRS on Bioko and to investigate whether the rise in malaria prevalence was related to the change in insecticide. Twenty four clusters of houses were randomly allocated to receive IRS with either bendiocarb or deltamethrin in 2014. Approximately three months after the intervention, prevalence of malaria infection and levels of haemoglobin were measured in children aged 2 to 14 in each cluster. Prevalence of infection was lower in the bendiocarb arm (16.8%, 95% CI 11.1 - 24.7, N = 1374) than in the deltamethrin arm (23.2%, 95% CI 16.0 - 32.3, N = 1330) but this difference was not significant ($p = 0.390$), even after adjusting for confounders ($p = 0.119$). Mean haemoglobin was significantly higher in the bendiocarb clusters (11.6g/dl, 95% CI 11.5 - 11.8, N = 1326) than in the deltamethrin clusters (11.5g/dl, 95% CI 11.3 - 11.7, N = 1329), ($p = 0.049$ after adjusting for confounders). The results of this study are somewhat inconclusive by themselves, but they suggest that, on Bioko, bendiocarb may offer better protection against malaria infection than deltamethrin. Subsequent data on phenotypic and metabolic resistance to pyrethroids of local vectors would suggest that pyrethroid resistance renders deltamethrin IRS ineffective for malaria control on Bioko.

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INSECTICIDE SUSCEPTIBILITY LEVELS OF *ANOPHELES GAMBIAE* S.L MOSQUITOES IN AWKA, NIGERIA

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Long-lasting insecticide nets (LLINs) and indoor residual spraying (IRS) are the main methods used for malaria vector control. However the success of these methods has been hampered by the development and spread of insecticide resistance in major malaria vectors. The emergence of insecticide resistance in *Anopheles* mosquitoes in Nigeria has enormous implications for vector control in the country. This study was therefore carried out to investigate the susceptibility status of *An. gambiae* s.l mosquitoes to the four main classes of insecticides used for vector control in Amansea community, Awka North LGA, Anambra State, Southeast Nigeria. Larval mosquitoes were collected from different breeding sites and reared in the insectary. Mosquitoes were identified morphologically and two to five day old adult female mosquitoes were used to conduct WHO susceptibility assays. Susceptibility assays were carried out against pyrethroids (0.75% permethrin and 0.05% deltamethrin), organochlorine (4% DDT), organophosphate (0.25% pirimiphos-methyl) and carbamates (0.1% bendiocarb and 0.1% propoxur) insecticides. All mosquitoes collected were identified as members of the *Anopheles gambiae* s.l. Susceptibility assays showed that the mosquitoes were completely susceptible to bendiocarb (100% mortality). Percentage mortality recorded for the other insecticides were as follows: DDT (1.3%), pirimiphos-methyl (15.6%), permethrin (26.3%), deltamethrin (38.8%) and propoxur (87.5%) respectively. The KDT_{50} recorded were bendiocarb (36.7 minutes), propoxur (39.8 minutes), deltamethrin (50.9 minutes), permethrin (91.4 minutes), Pirimiphos-Methyl (116.3 minutes) and DDT (119.1 minutes). On the other hand, the KDT_{95} recorded were bendiocarb (52.3 minutes), propoxur (62.3 minutes), deltamethrin (85.6 minutes), permethrin (171.1 minutes), Pirimiphos-Methyl (185.3 minutes) and DDT (170.1 minutes). The results show that there is very high frequency of insecticide resistance in the study area and calls for prompt implementation of resistance management practices in the area.

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THE EFFECT OF IVERMECTIN ON THE AMAZONIAN MALARIA VECTOR *ANOPHELES DARLINGI*: LC_{50} DETERMINATION

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Current malaria vector control measures target mainly endophagic *Anopheles* vectors; yet malaria transmission in South America is dominated by exophagic and exophilic vectors, indicating the need to evaluate novel control tools effective against such vectors. Ivermectin mass drug administration has been shown to be lethal to wild *Anopheles* in West Africa and has the potential to target exophagic and exophilic vectors. Although lethal to numerous *Anopheles* vectors, there is no information on the effects of ivermectin on South American vectors. Here, we evaluated the impact of ivermectin on the dominant Amazonian malaria vector, *An. darlingi* through *in vitro* experiments. We estimated the lethal concentration of ivermectin that kills 50% (LC_{50}), 25% (LC_{25}) and 5% (LC_5) of mosquitoes in membrane feeding experiments. Varying concentrations of ivermectin were blood fed to 3-5 day old, laboratory-reared (F_{23}-F_{29}) *An. darlingi* mosquitoes; survivorship was monitored and recorded daily for 7 days post blood meal. Ten replicates with four to ten ivermectin concentrations per replicate, including a control, were tested across a range of 4 – 1000 ng/ml. The LC_{50}, LC_{25}, and LC_5 of ivermectin fed to *An. darlingi* was calculated as LC_{50} = 36.4 ng/ml [29.7, 42.7], LC_{25} =20.7 ng/ml [14.1, 26.1], and LC_5 = 9.2 ng/ml [4.5, 13.7] (n = 4333, 10 replicates). These LC_{50} values demonstrate that *An. darlingi* is susceptible to ivermectin concentrations found in humans post oral drug administration. Future *in vitro* experiments will determine if ivermectin inhibits the ability of *An. darlingi* to re-feed as was demonstrated in *An. gambiae* previously, and investigate whether ivermectin inhibits the development of *P. vivax* in *An. darlingi* as has been shown with *P. falciparum* in *An. gambiae* and more recently with *P. vivax* in *An. dirus*. *In vitro* ivermectin mosquito-lethal effects against *An. darlingi* would suggest that ivermectin mass drug administration could be a novel, powerful malaria vector control tool in Peru and other Amazonian countries.

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USING STABLE ISOTOPES OF CARBON AND NITROGEN TO MARK WILD POPULATIONS OF *ANOPHELES* AND *AEDES* MOSQUITOES IN SOUTHEASTERN TANZANIA

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Marking wild mosquitoes is important for understanding their ecology, behaviours and role in disease transmission. Traditional insect marking techniques include using dyes, biological agents and tags but such techniques have various limitations like low marker retention and inability to mark wild mosquitoes at source. Stable isotopes are gaining wide spread use for non-invasive marking of arthropods, permitting greater understanding of mosquito dispersal and responses to interventions. We describe here a simple technique for marking naturally-breeding malaria and dengue vectors using stable isotopes of nitrogen (15N) and carbon (13C), and describe potential field applications. We created man-made aquatic mosquito habitats and added either 15N-labelled potassium nitrate or 13C-labelled glucose, leaving non-adulterated habitats as controls. We then allowed wild mosquitoes to lay eggs in these habitats and monitored their development in situ. Pupae were collected promptly as they appeared and kept in netting cages. Emergent adults (in pools of ~4 mosquitoes/pool) and individually stored pupae were desiccated and analysed using Isotope Ratio Mass Spectrometry (IRMS).*Anopheles gambiae* s.l and *Aedes* spp. from enriched 13C and enriched 15N larval habitats had significantly higher isotopic levels than controls (P=0.005), and both isotopes produced sufficient distinction between marked and unmarked mosquitoes. Mean δ15N for enriched females and males were 275.64±65.12 and 247.95±54.55, while mean δ15N in controls were 2.1±0.1 and 3.9±1.7 respectively. Similarly, mean δ13C for enriched females and males were 36.08±5.28 and 38.5±6.86, compared to -4.3±0.2 and -7.9±3.6 in controls respectively. In all cases, there were variations in standardized isotopic ratios between mosquito species. Enrichment of semi-natural mosquito larval habitats with stable isotopes of nitrogen and carbon resulted in effective marking of *Anopheles* and *Aedes* mosquitoes colonizing these habitats. This approach can significantly enhance studies on mosquito eco-physiology, dispersal, pathogen transmission and responses to control measures.

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THE ROLE OF IMMUNE PATHWAYS IN *WOLBACHIA*-MEDIATED BLOCKING OF DENGUE VIRUS IN *AEDES AEGYPTI*

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The bacterial endosymbiont *Wolbachia pipientis* has been shown to protect its host against many pathogens, including dengue virus (DENV). DENV's main vector, *Aedes aegypti*, is not a natural carrier of the bacterium but a stable transinfection was achieved some years ago using the *Wolbachia* strain *w*Mel. Due to the protection conferred by *Wolbachia*, the symbiont is being developed as a means of vector control to reduce disease incidence long-term. Although, *Wolbachia*-induced priming of the immune system has been suggested as a mechanism conferring pathogen protection its role is poorly understood. Here we attempted to determine the individual contribution of several insect immune pathways to *Wolbachia's* protective ability. Using RNAi techniques, knockdown of genes representing each of the five major insect immune pathways (TOLL, Imd, Autophagy, JAK/STAT and RNAi) was achieved in *A. aegypti* cells (+- *w*Mel). Simultaneous knockdown of more than one gene was also performed to assess for multifactorial effects. Cells were challenged with a DENV-2 strain after the manipulation of host immune gene expression and DENV copy number was evaluated 5 days post-infection. We found that *w*Mel based blocking was dependent on 2 of the pathways; JAK/STAT and RNAi pathways. Both pathways are known to be involved in the antiviral response and are known to be manipulated by dengue virus infection. A weaker interaction was found between *Wolbachia* and the Toll and Autophagy, but not Imd pathways. Our results suggest that both the JAK/STAT and RNAi pathways are the main immune contributors to *Wolbachia*-mediated DENV blocking, since suppression of its effectors lead to a dramatic increase in the dengue virus titre.

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COMPARISON OF MORPHOLOGICAL AND MOLECULAR IDENTIFICATION OF OUTDOOR ANOPHELINE MOSQUITO SPECIES IN AN AREA TARGETED FOR ELIMINATION IN SOUTHERN ZAMBIA

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Malaria was once a leading cause of morbidity and mortality in Macha, Choma District, Southern Zambia. However, in the past decade malaria incidence has declined and elimination strategies are now being rolled out. In conjunction with this decline, few numbers of the primary local malaria vector, *Anopheles arabiensis* caught indoors are still present in the vector population. Malaria cases are reported at health facilities with severe cases and deaths recently rising at Macha Hospital. To investigate the contribution that other anopheline species that forage outdoors make to malaria transmission, collections from homesteads within the catchment area of Macha Hospital were conducted. Standard morphology in conjunction with molecular tools were used for identification of mosquitoes caught between February 2015 and April 2015 from UV traps set outdoors next to animal enclosures. A total number of 1283 mosquitoes were collected of which 650 (50.7%) were anophelines. Morphological identifications revealed seven different species. Following DNA extractions of the abdomen, PCR was employed which amplified the intergenic-spacer -2 region of the nuclear rDNA. Of the 640 samples successfully analyzed, 11 different anopheline species were identified molecularly with the majority (53.8%) being *An. squamosus*. Morphological identification of specimens accurately identified 85% of *An. squamosus*, and 62% of the second been *An. arabiensis*. As malaria control targets and impacts the major malaria vectors in Zambia, surveillance of previously understudied species is important as they begin to constitute a larger proportion of potential vector collections. The use of molecular-based techniques for the identification of anopheline mosquitoes is vital to confirm identities, assign behaviors to particular species and accurately determine each species contribution to malaria transmission.

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IS *ANOPHELES PALUDIS* A VECTOR OF MALARIA IN THE DEMOCRATIC REPUBLIC OF CONGO?

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Anopheles paludis was the most common *Anopheles* mosquito collected in human landing collections in Lodja, central DRC, during sentinel site collections in 2013 and 2014. *Anopheles paludis* had been previously collected in the neighboring province of Bandundu with sporozoite rates of 6%. To see if *An. paludis* was acting as a vector in Lodja, monthly human landing catches and pyrethrum spray catches were conducted over the course of 2015. Mosquitoes were identified to species and *Anopheles* mosquitoes were tested for presence of circumsporozoite

protein using ELISA. *An. paludis* was the most common I mosquito collected in 2015. *An. gambiae* s.l. and *An. funestus* were also collected. *An. paludis* displayed an early biting peak, with the highest numbers collected between 1900 and 2000h. None of 1366 *An. paludis* females tested positive for sporozoites. Despite high densities of anthropophilic *An. paludis* present in Lodja, it does not appear to be an important vector of malaria. Further work is needed to understand whether there are differences between *An. paludis* populations in Lodja and Bandundu.

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DOMINANT ROLE OF *ANOPHELES FUNESTUS* GILES, IN A RESIDUAL TRANSMISSION SETTING IN SOUTHEASTERN TANZANIA

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Malaria is transmitted by more than one anopheline mosquito species, but the roles of these species vary in different epidemiological settings. We assessed the role of the two dominant *Anopheles* mosquito species in selected villages in Ulanga and Kilombero districts in south-eastern Tanzania. Monthly mosquito sampling was done in randomly selected households in three villages using CDC light traps, back pack aspirators and HLC between January -2015 and January-2016. Multiplex polymerase chain reaction (PCR) was used to identify members of the *An. funestus* group mosquitoes and *An. gambiae* s.l, to species level. Enzyme-linked immunosorbent assay (ELISA) was done to detect *Plasmodium* sporozoites in the mosquito salivary glands, and to identify sources of mosquito blood meals. The geographical distribution of infected *An. funestus* and *An. arabiensis* mosquitoes was determined by ArcGIS 10 (ESRI-USA) software. A total of 22,391 *An. arabiensis* and 4,802 *An. funestus* were collected. Among the *An. funestus* group mosquitoes, *An. funestus* s.s. predominated (76.6%), An. rivulorum (2.9%) and An. leesoni (7.1%) and unamplified samples (13.4%). About 86% of all infected mosquitoes were *An. funestus* s.s. while 14% were *An. arabiensis*. Overall, *An. funestus* group contributed to 93.4% and *An. arabiensis* contributed to 5.6% respectively of the annual EIR. In the *An. funestus* group, *An. funestus* s.s contributed to 96% of the transmission while An. rivulorum contributed 4%. Mosquito blood meal sources included: humans, 79.2%, bovine, 17.1%, dog, 2.4% and chicken, 1.2%. Infected *An. funestus* were distributed across the study area while infected *An. arabiensis* were confined to small part in the middle of study area. Though *An. arabiensis* are still the most prevalent of the vector species in the study area, ongoing residual malaria transmission could is predominantly mediated by *An. funestus*. The evidence from this study demonstrates that *An. funestus* have a significant role as the main driver of malaria transmission in these study villages. *An. funestus* could be responsible for resurging malaria transmission in communities where LLINs are widely distributed.

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COMPARATIVE FLAVIVIRUS SUSCEPTIBILITY AMONG *AEDES AEGYPTI* STRAINS UNDER LABORATORY AND SIMULATED FIELD CONDITIONS

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The mosquito *Aedes aegypti* is the primary global vector of dengue virus and Zika virus. Previous investigations have demonstrated variable levels of virus susceptibility, and subsequently vector competence, due to genetic variability between *Aedes aegypti* strains. However, previous studies uniformly employed optimal laboratory mosquito rearing conditions that do not reflect the effects of environmental variability encountered by larvae under natural conditions. Our previous studies have shown significant differences in body size between field vs laboratory reared individuals of the same genetic background. Here, we characterized and compared differences in susceptibility of three *A. aegypti* laboratory strains and a recent *A. aegypti* field isolate from Trinidad to dengue virus (DENV2 JAM1409) and two isolates of Zika virus (Zika CAM, Zika MAL) under both optimized laboratory regimes and conditions simulating realistic, nutrient deficient field habitats. We present dissemination rates of each mosquito strain to each virus as well as quantify viral load in female *A. aegpyti* mosquitoes 14 days post infection. The implications of gene by environment interaction, and the role of phenotypic plasticity are then further discussed in the contexts of capitalizing on increasing our knowledge on heritable differences in flavivirus susceptibility to better inform disease prevention programs.

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EVALUATING NEW TOOLS FOR MONITORING BRAZILIAN AND EAST AFRICAN MALARIA VECTORS

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To sufficiently monitor ongoing malaria transmission and evaluate whether interventions are achieving desired effects, adequate mosquito surveillance methods are essential. It is important that such tools are applicable in several environments and different countries, rather than just in specific localities. This study aimed to assess the BG-Malaria trap (BGM), which has recently been demonstrated as effective against the Brazilian malaria vector, *Anopheles* dalingi, as a practical method for monitoring malaria vectors also in east Africa. We compared BGM with BG-Sentinel trap (BGS) and Human Landing Catches (HLC) for sampling African malaria mosquitoes, using a set of two separate 3 by 3 Latin square experiments replicated 4 times each. The field study was conducted in rural Ulanga district, in southeast Tanzania for 12 nights (06:00pm - 06:00am). Separately we evaluated 5 different mosquito lures, all using BGM and parity ratios were assessed for all traps and compared. We collected a total of 1003 *Anopheles* mosquitos, almost half of whichit were caught by BGM (49.7%). HLC and BGS caught 34.0% and 16.3% respectively, with a significant difference between the methods (P=<0,0001). The mean numbers of *An. gambiae* and *An. funestus* caught in the BGM per night were19.0 [CI: 17.45 - 20.96] and 3.5 [2.22 - 5.49]. HLC caught 11.7 [CI: 10.09 - 13.58] and 1.3 [0.68 - 2.44] and BGS caught 6.0 [CI: 4.60 - 6.76] and 1.2 [0.62 - 2.24] respectively. The others *Anopheles* species corresponded to 2% of total mosquitos caught. Data on the comparative evaluation of the lures, and also on parity status of the mosquitoes is being analysed and will be presented at the meeting. The BGM trap was considerably more effective than BGS, a trap widely used for monitoring vectors, and performed better as HLC as well, which is considered the "gold standard" method for catching mosquitos. The results described here for BGM are promising and hence could be used as a surveillance tool for malaria vectors. More tests are underway to validate the results in other sites in the district and will be presented at the meeting.

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EVALUATION OF A NOVEL POLYTETRAFLUOROETHYLENE (P.T.F.E) - BASED MEMBRANE FOR BLOOD-FEEDING MALARIA AND DENGUE FEVER VECTORS

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Blood feeding of female mosquitoes is an essential activity for colonization and maintenance of mosquitoes which are often required for research on vector-borne diseases. Common laboratory blood feeding strategies for rearing mosquito colonies use direct host feeding (DHF) such as on human arms or on live animals. The aim of this study was to evaluate the

artificial way of blood-feeding mosquitoes which is simple, affordable, sustainable and efficient in comparison to existing method DHF. We adapted and validated an artificial feeding (AF) method to replace DHF for the maintenance of *Aedes aegypti*, *Anopheles arabiensis* and *An. gambiae* s.s mosquito colonies. This blood feeding system uses fresh bovine blood as blood meal source, stored in vacutainer tubes containing the anticoagulant, ethylenediaminetetraacetic acid (EDTA). The blood was placed at the bottom surface of the disposable styrofoam cups and a simple membrane made of polytetrafluoroethylene (P.T.F.E) was stretched thinly over the bottom surface of the cup for mosquitoes to access the membrane and were fed for 20 min. Blood feeding rate, fecundity and survival rates of mosquitoes fed using the AF were compared to those mosquitoes fed using direct human arm feeding.The preliminary results show that *Ae. aegypti* mosquitoes had similar feeding rates on AF and DHF of about 100%. However the feeding rates of *An. arabiensis* and *An. gambiae* s.s on DHF had the highest rates compared to membrane feeding. Artificial feeding rates on *An. arabiensis* and *Anopheles gambiae* were (40%, 47.5%) and (52%, 48.5%) for the first and second replicate respectively while DHF the feeding rate was about 99% in malaria vectors for both replicates. The results show that bovine blood meal source has impact on the feeding rates of malaria vectors the *An. gambiae* s.s and *An. arabiensis* in comparison to *Ae. aegypti* species. Data of fecundity and survival will be presented at the meeting.The procedure can be adopted by laboratories due to its simple and affordable materials and design and it could be used as an alternative to the direct feeding method in different biological studies and studies on parasite transmission by vectors.

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JAPANESE ENCEPHALITIS IN SIVASAGAR, ASSAM, INDIA, 2011-2014

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Japanese encephalitis (JE) is a vaccine-preventable cause of acute encephalitis syndrome (AES) with high (20-30%) case fatality rate (CFR). India has a high JE global burden;however, 50% JE cases are in Assam state. We estimated JE burden by time, place, and person in Sivasagar district, a high burden district within Assam, from 2011-2014. We reviewed records and reports of all AES patients who attended three hospitals in Sivasagar from January 1, 2011 to December 31, 2014. We evaluated AES/JE by time, place, and person, including disease trends and patients' vaccination status.In 2011-2014, Assam had 7,498 AES patients of which 647 (9%) were from Sivasagar (2011: 246; 2012: 140; 2013: 133; and 2014: 128). All were tested for JE virus and 314 (49%) were positive (2011: 128, 52%; 2012: 58, 41%; 2013: 64, 48%; and 2014: 64, 50%). During 2011-14, the majority of JE (291/314, 93%) and AES (313/333, 94%) patients were from rural areas and most JE patients were reported in July (65%). Median age of JE patients was 43 years (0.9-97 years) and AES was 29 years (0.1-85 years). Among JE patients, males (62%) were more affected whereas among AES, gender proportions were similar (males: 51%). Among 647 patients, 54 AES (16%) and 29 JE (9%) patients were vaccinated against JE. The CFR of AES and JE were similar (AES:38%; JE:38%) in Sivasagar and were both higher than the Assam average (AES: 17%; JE: 23%). JE/AES in Sivasagar with a low vaccination coverage and high CFR calls for urgent public health attention especially for rural areas and older persons. Analysis of risk factors and prevention strategies is recommended.

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THE IMPACT OF SOCIODEMOGRAPHIC FACTORS ON *AEDES ALBOPICTUS* DISTRIBUTION AND ABUNDANCE IN ATLANTA, GEORGIA

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Since its invasion to the continental United States in 1985, the *Aedes albopictus* mosquito has become a well-established nuisance mosquito. Breeding in both natural and artificial containers, the distribution of this mosquito may be strongly influenced by humans. Although considered a secondary vector of certain arboviruses, recent outbreaks have caused its vector status to be reevaluated. Determining the sociodemographic factors which affect the distribution of this mosquito throughout distinct geographical locations is paramount in limiting future establishments and preventing the transmission of pathogens. A total of 142 houses were sampled across three neighborhoods with varying house values in Georgia, USA between July and August 2015. Non-parametric tests were utilized to determine if differences existed between container type and the three neighborhoods. The importance of house value was assessed within neighborhoods using simple linear regression. Finally, negative binomial generalized linear models with and without random effects were created in order to identify significant predictors of *Ae. albopictus* and container abundance across the three neighborhoods. The median number of rubber containers differed significantly between the low house value neighborhood and the high and middle neighborhoods (p-value = <0.001 and p-value= <0.001, respectively). None of the simple linear regressions yielded significant results; however, clear associations were present. The Akaike weights from the model which best accounted for number of mosquito-positive containers was 35.3% and IV instar *Ae. albopictus* larvae was 41.5%. House value was determined to be either the best predictor or one of the best predictors in the chosen models. The non-parametric tests and simple linear regressions suggest that house value does impact breeding site abundance and *Ae. albopictus* distribution. The multivariate hierarchical models support the hypothesis that the distribution of *Ae. albopictus* larvae depends, at least in part, on sociodemographic factors.

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COMPARATIVE EVALUATION OF SIX OUTDOOR SAMPLING TRAPS FOR DISEASE- TRANSMITTING MOSQUITOES IN REFERENCE TO HUMAN LANDING CATCH IN RURAL TANZANIA

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There is a growing concern on how mosquito sampling methods can be safely performed in malaria endemic countries. Human landing catch is the best mosquito sampling method, it is labor intensive and exposes induvial to malaria transmission risks. This is an ongoing study which assesses the different mosquito sampling traps aiming to find an alternative for HLC in terms of effectiveness, densities, diversities and behaviors of disease- transmitting mosquitoes.Seven traps were introduced to sample mosquitoes. These were Mosquito Magnet (MMX), BG-Sentinel, Suna trap, Ifakara Tent Trap-C (ITT-C), M-Trap and M-Trap fitted with CDC Light trap. The traps were comparatively evaluated and calibrated with reference to the Human Landing Catch (HLC). A series of multiple 7*7 latin square experiments were conducted in 6 different villages over a total of 12 months, working in dry and wet season in each of the villages, to comparatively evaluate 6 different trap types against human landing

catches, the current gold-standard sampling tool. Seven position identified to each of this villages with the distance of 100m from one trap to another. The different traps rotated to the seven positions, that at the end of a 7 day rotation, each trap type had been to each of the seven locations at least once. The experiment were replicated 3 times for a total of at least 21 nights, start from 18:00hrs to 06:00hrs. The outcome measure will be the comparisons of effectiveness of different traps in terms of capturing high density and diversities of outdoor host seeking mosquitoes relatively to the reference method (HLC).A total of 62317 of all female mosquitos were collected for six villages for both seasons wet and dry, where BG-Sentinel n=5571 (8.94%), HLC n=13909 (22.32%) ITT-C n= 3775 (6.06) MMX n= 4468(7.17%) M-Trap n= 8429 (13.52) M-Trap with CDC Light trap n=12011(19.27%) and Suna trap n=14003 (22.47%) The result is showing there is no significant different between HLC, Suna trap and M-trap fitted with CDC for all total number of female mosquitoes but there is a different between HLC, against M-trap, BG-Sentinel, MMX and ITT-C in the first round.

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CHARACTERIZATION OF SURFACE LAYER MICROBIAL COMMUNITIES OF *ANOPHELES GAMBIAE* COMPLEX LARVAL HABITATS IN BURKINA FASO

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Afro-tropical malaria vectors of the *Anopheles gambiae* complex represents a remarkable example of adaptive radiation thought to be driven by ecological divergence operating predominantly on the larval stages. Some ecological factors involved in larval niche partitioning, such as different ability to escape predators or tolerate abiotic stress, have been identified. Little is known, however, about the role in this process of environmental microbial communities occurring in the larval habitats. Thus, we tested the hypothesis that members of the *An. gambiae* complex are preferentially associated with different microbial communities.To this aim, we sampled *An. gambiae* s.l. larvae and the surface layer of 63 randomly chosen water collections in the village of Goundry (Burkina Faso). The microbiological profile of each site was obtained by PCR amplification using consensus primers flanking the V6-V8 region of bacterial 16S rDNA, and subsequent sequencing by Illumina Miseq. Paired-end reads were taxonomically analysed using the two bioinformatic pipelines BioMaS, for identification at species level, and QIIME, for OTU based analysis. The relative frequencies of mosquito species occurring in the larval sites (i.e. An. arabiensis, *An. gambiae*, and *An. coluzzii*) were associated to the inferred bacterial composition by Canonical Correspondence Analysis (CCA).Preliminary analysis of a subsample of 37 breeding sites based on 1,620 molecularly-identified mosquitoes (45% *An. coluzzii*, 38% An. arabiensis, 17% *An. gambiae*) showed that bacterial composition accounted for 6% of the total variance in larval relative frequencies. The first two canonical axes, which accounted for ~75% of the explained variance, separated the three species and associated bacterial communities.The results indicate that microbial communities occurring in larval habitats can be informative about the composition of sympatric species of the *An. gambiae* complex, supporting the hypothesis that particular environmental bacteria may represent an ecological marker of niche partitioning for these malaria vector species.

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A DRAMATIC DECLINE OF MALARIA TRANSMISSION IN AND AROUND IFAKARA, A RAPIDLY GROWING TOWN IN SOUTHEASTERN TANZANIA, SINCE 2000

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Ifakara is a small but rapidly growing town of about 60,000 people in South-eastern Tanzania. A steady but high increase of population in Ifakara area has resulted in a rapid urbanization of the area, which in turn has had a negative impact on malaria transmission. In 2003, Ifakara had an estimated annual entomological inoculation rate (EIR) of 29. Our study aimed at determining changes in malaria transmission over the past decade. A total of 110 households were randomly sampled from across the five wards of Ifakara area. Mosquito collection was done between June 2015 and January 2016, using CDC light traps indoors, and Suna® traps outdoors. Comparison of indoor and outdoor mosquito density was done using the Human Landing Catches (HLC). *Anopheles* mosquitoes were morphologically identified, and analysed for *Plasmodium* sporozoites. Blood fed mosquitoes were also examined for blood-meal sources. A total of 2658 *Anopheles* mosquitoes were caught from 800 trap nights and 80 Human Landing Catches, including: 2,131 *Anopheles gambiae* sensu lato, 355 *Anopheles funestus* group, and 172 *Anopheles* coustani. Of all the malaria vectors, 85% were collected only from two wards, which were the most rural of the 5 Ifakara wards. All the *An. gambiae* s.l. were identified as An. arabiensis, and 95% of the *An. funestus* were identified as *An. funestus* funestus, the rest being An. rivulorum. Enzyme-linked immunosorbent assays were performed on 2,658 *Anopheles* mosquitoes and only one was found positive, which was an *An. funestus* caught outdoors by HLC in Katindiuka ward. *Plasmodium* sporozoite rate was calculated as 0.04% in all *An. gambiae* and *An. funestus* combined, and 2.8% in just the *An. funestus*. Overall mean nightly biting rates by malaria vectors were 3.02 mosquitoes per night, thus the EIR was estimated as 0.128. In conclusion, the EIR in Ifakara has dropped by over 99% in just over a decade, compared to what was observed in previous reports. The on-going transmission is concentrated in only a small and more rural section of the Ifakara area, which could be readily targeted with improved control measures towards local elimination.

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SPATIAL SEGREGATION OF VECTOR MOSQUITOES IN URBAN BALTIMORE, MD

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Understanding processes that govern vector mosquito coexistence can help predict disease risk and guide public health interventions. Theoretical and empirical ecology indicate that under resource-limiting conditions in a constant environment, competition between species should result in the exclusion of the inferior competitor. Multiple vector mosquito species coexist in southwest Baltimore, MD, where vacant lots containing high densities of water-filled trash containers are interspersed within a matrix of maintained lots with fewer containers. One hypothesis that may help explain the coexistence of *Culex* mosquitoes with the superiorly competitive *Ae. albopictus* in Baltimore is a colonization-competition tradeoff, which predicts species coexistence in an environment with ideal habitats when an inferior competitor in a metapopulation can escape exclusion by having a superior ability to colonize vacant or sparsely populated patches. In this study, we tested the prediction that there would the highest abundances of *Cx.* species and *Ae. albopictus* in vacant lots compared to intervening occupied lots. We placed 5 oviposition traps in each of 6 vacant lots and 6 randomly selected sites in intervening

maintained lots in early and late mosquito season in paired city blocks in southwest Baltimore. Resident containers at each site were also surveyed for mosquito larvae during the late season session. In the early season, *Cx.* species and *Ae. albopictus* made up 80.8% and 15.2% of the 2619 total collected larvae, respectively. 75% of early season larvae came from abandoned lots. In the late season, *Culex* species and *Ae. albopictus* comprised 16.8% and 83.2% of larvae, respectively. 45 resident containers were identified with the majority (36) found in vacant lots. 51% of the resident containers contained larvae, with 87% of the larvae coming from abandoned lots. Resident container data and early season ovitrapping support the prediction that vacant lots support both higher abundances of larval habitat and abundances of *Culex* and *Ae. albopictus* compared to maintained lots, and should be targeted for mosquito reduction interventions in the future.

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EXPERIMENTAL PERTURBATIONS OF *CULEX RESTUANS* POPULATIONS AND THEIR EFFECT ON WEST NILE VIRUS TRANSMISSION BY MEMBERS OF THE *CX. PIPIENS* COMPLEX

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In the eastern United States, the mosquito species *Culex restuans* and members of the *Culex pipiens* complex transition in abundance and epidemiological importance as vectors of urban arboviruses. *Cx. restuans* is most active during periods of West Nile virus (WNv) reemergence (early season) and the *Cx. pipiens* complex is most active during periods of peak WNv transmission (summer) and is considered the primary vector of WNv in the U.S. Previous studies have suggested evidence for early season enzootic WNv transmission by *Cx. restuans*, yet an empirical connection between the two species' transmission networks has not been established. We designed a semi-natural treatment-control experiment to test the hypothesis that the seasonal reemergence of the enzootic WNv transmission cycle is linked to the presence and blood feeding activities of early season *Cx. restuans* populations. To test this hypothesis, from March 21st to June 1st, 2016 we applied a rotating combination of methoprene (8.62%) and Bacillus thuringiensis (Bti) (10.31%) larvicides in road-side catch basins and storm drains in two urban parks in Atlanta, GA at temporal intervals corresponding with peak abundance of *Cx. restuans* populations. We then monitored WNv infection in the enzootic cycle during and post treatment by 1) testing blood samples obtained from the resident passerine birds for evidence of WNv antibodies and 2) testing all *Culex* spp. mosquitoes collected with aspirators, gravid traps, and CDC light traps for WNv infections. These surveillance methods were paired with collections from two untreated parks in Atlanta. Preliminary Before-After Control Intervention analyses show that *Cx. restuans* breeding populations were successfully suppressed in the treatment parks relative to the controls; however there was no effect of larval control on adult *Cx. restuans* population abundance. Avian WNv antibody seroprevalence and *Culex* spp. mosquito WNv infection data are currently being processed.

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PRESENCE OF SPECIES WITHIN THE *ANOPHELES GAMBIAE* COMPLEX IN THE DEMOCRATIC REPUBLIC OF CONGO

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Anopheles gambiae s.l. is the primary vector of malaria in the Democratic Republic of Congo, however, there is little data on the species from this complex present in the country. This paper presents the species collected (as determined by PCR) between 2004 and 2011 in 16 locations across the country. The two species from the *An. gambiae* complex that were detected were *An. coluzzii* and *An. gambiae* s.s. *An. gambiae* s.s. was predominant in eastern DRC, whereas *An. coluzzii* was the main species found in several locations in Bandundu. The species were also found in sympatry in several locations (Kinshasa, Kisangani, Lodja). These results provide a basis for future work, which is needed to accurately describe the distribution of the *An. gambiae* complex species in DRC.

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MAPPING PAST, PRESENT, AND FUTURE CLIMATIC SUITABILITY FOR INVASIVE *AEDES AEGYPTI* AND *AE. ALBOPICTUS* IN THE UNITED STATES: A PROCESS-BASED MODELING APPROACH

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Rapid changes in the distributions of the mosquitoes, *Aedes aegypti* and *Ae. albopictus* in the continental United States alter the potential for local transmission of dengue, chikungunya, and Zika viruses. All three viruses have caused major disease outbreaks in the Americas recently with infected travelers returning regularly to the U.S. Recent outbreaks of Zika, dengue, and chikungunya have proven that these diseases are capable of invading and being transmitted within the ranges of *Ae. aegypti* and *Ae. albopictus,* mostly in warm and tropical regions. The expanding range of these mosquitoes and discovery of new populations within the U.S. raises questions about whether recent spread has been enabled by climate change or other anthropogenic influences. In this analysis, we used daily average temperatures for the United States to model *Ae. aegypti* and *Ae. albopictus* population growth rates using a stage-structured matrix population model in order to understand past and present habitat suitability of these vectors, and to project future habitat suitability under IPCC climate change scenarios. We applied our model to the continental U.S. using temperature data on a 4-km grid. Our results indicate that, as expected, much of the southern U.S. is suitable for both *Ae. aegypti* and *Ae. albopictus* year-round; however, a surprisingly large proportion of the U.S. in addition to the southern states is suitable for positive population growth for much of the year. While the amount of suitable habitat in some regions of the U.S. has contracted within the past 50 years, the range of suitable habitat for both *Ae. aegypti* and *Ae. albopictus* has expanded in many regions across the country. Additionally, IPCC CMIP5 model projections of future climate change suggest that climate change will reshape the range of *Ae. aegypti* and *Ae. albopictus* in the U.S., and potentially the risk of the viruses they transmit. Understanding the range of these mosquitoes should be considered a high priority for public health officials and vector control agencies.

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ABUNDANCE OF *AEDES ALBOPICTUS* AND PRESENCE OF DENGUE VIRUS IN PROXIMITY TO A PINEAPPLE PLANTATION IN COSTA RICA

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The role of *Aedes albopictus* in the transmission of dengue viruses (DENV) in the Americas is unknown. Considering that development of larvae can occur in bromeliads, the aim of this study was to determine the abundance of *Ae. albopictus* at three sites in proximity of an organic pineapple plantation, as well as to evaluate the presence of DENV. For adult collections, CO_2 traps were placed for 20-24 hrs in forested areas and houses adjacent to the plantation, as well as houses >1 km from the plantation. Pineapple plants were evaluated for mosquito larvae or pupae (8 clusters of 40 plants), as well as containers in and around 27 houses adjacent and 29 far from the plantation. Pools of *Ae. albopictus* were analyzed by RT-PCR, and DENV serotype was identified by sequencing. Adult mosquitoes at all sites included mainly *Anopheles apicimacula*, *Culex coronator*, *Cx. quinquefasciatus*, *Cx. nigripalpus*, *Cx. inflictus*, and *Ae. albopictus*. Biodiversity index (Shannon-Wiener) was higher in forested areas (1.39) than in houses adjacent (1.1) or far (0.75) from the plantation. *Cx. nigripalpus* and *Cx. quinquefasciatus* were the most abundant species in the forested area and houses, respectively. Adult *Ae. albopictus* were more common in forested areas and adjacent houses. Only 2 mosquito larvae were collected from pineapple plants. *Ae. albopictus* was the most frequent species in containers from houses; although house (HI) and container (CI) indices were higher in houses farther from the plantation (HI: 51, CI: 49) than those adjacent to it (HI: 41, CI: 29). DENV-2 and DENV-3 were detected in 2 of 20 pools of *Ae. albopictus* heads, and DENV-1 in 2 of 74 pools of larvae. Results suggest that nearby forested areas, which provide a natural habitat, may be the preferred sites for *Ae. albopictus*. However, the abundance and types of breeding sites in these forested areas is still to be determined. Moreover, results confirm that local *Ae. albopictus* harbor DENV, and that horizontal transmission is occurring. Further investigations will be required to determine the source of DENV and weather mosquitoes in forested areas are biting humans, monkeys, or other animals.

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FLIGHT APTITUDE OF TETHERED MOSQUITOES AS A MEASURE FOR LONG DISTANCE MIGRATION BEHAVIOR

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Malaria kills over 500,000 people every year in sub-Saharan Africa. During the dry season in the Sahel, surface water required for larval sites disappears from this vast region, halting mosquito reproduction and bringing malaria transmission almost to a standstill. Recent studies have suggested that both *Anopheles gambiae* s.s and *An. arabiensis* (but not *An. coluzzii*) persist in this region by migrating from distant locations, where breeding occurs year round, though direct evidence for long-distance migrating malaria vectors to date is scant. In many insects worldwide, windborne long-distance migration occurs seasonally, facilitating exploitation of renewed resources far beyond their locomotor capabilities. In this study, our aim was to measure flight behavior in stationary-tethered, wild mosquitoes, and evaluate if flight intensity exhibits bimodality, consistent with "weak" and "strong" flying mosquitoes. Additionally, we evaluated the seasonal variation in flight behavior and compared it to the expected migration time of each species. Mosquito flight sound was recorded individually over 10 hour experiments. This assay enables us to characterize flight behavior of individual mosquitoes controlling for species, sex, and physiologic state. In the laboratory, this assay revealed the effect of age on flight aptitude. Preliminary results on data obtained using the assay on wild mosquitoes in Mali since July 2015 suggest that flight aptitude exhibits a wide variance among species and over seasons. Flight aptitude was elevated a few weeks after the first rains, consistent with results obtained using our free-flight assay (see poster), and in agreement with season-specific long-distance-migration. Comprehensive analysis of year-long data will be presented.

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THE RELATIONSHIP BETWEEN ENTOMOLOGICAL INDICATORS OF *AEDES AEGYPTI* ABUNDANCE AND DENGUE INFECTION

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Routine entomological monitoring is a method to identify individuals at risk of dengue virus (DENV) infection. Using longitudinal entomological and serological data from Iquitos, Peru, we estimated the six-month risk of DENV for several *Aedes aegypti* monitoring indicators to determine whether *Ae. aegypti* abundance measures are associated with human DENV infection. Entomological survey data were linked with 8,157 paired serological observations taken approximately six months apart. Indicators of *Ae. aegypti* density were calculated from cross-sectional entomological data and linked by date to serological observations. Risk ratios estimating the association between *Ae. aegypti* abundance at the household and block levels and the six-month risk of dengue virus (DENV) seroconversion were obtained using log binomial models in a generalized estimating equation to control for repeated measures and clustering due to household membership. Risk ratios estimated using cross-sectional data were compared to risk ratios estimated from density measures calculated with entomological data collected up to 12 months prior to the start of the seroconversion interval. Cross-sectional *Ae. aegypti* densities (adult and immature mosquitoes) were not associated with an increased risk of DENV seroconversion. Larval and pupal measures showed no difference in risk. Incorporation of up to 12 months of prior entomological data into density estimates resulted in plausible risk ratio estimates for adult stage measures at the household and block levels, with adjusted risk ratios ranging from 1.02 (95% CI: 1.01,1.02) to 1.39 (95% CI: 1.17, 1.66), which was the strongest association with DENV seroconversion. Our results are likely due to the temporal variability in adult *Ae. aegypti* measures that result in areas with low levels of infestation being misclassified as having no entomological risk.

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ADEQUACY OF DIETARY DIVERSITY FOR YOUNG CHILDREN IN THE DOMINICAN REPUBLIC AS A FUNCTION OF CHILD AGE AND HOUSEHOLD WEALTH

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Dietary diversity is one critical domain indexing the adequacy of children's diets. However, research to date has not adequately examined deficits in dietary diversity by child age despite marked changes in diets and dietary needs that occur over the first several years of life. Such information may guide the tailoring of nutrition education by child age. The aim of this study was to determine the extent and nature of deficits in dietary diversity as a function of (i) age of young children and (ii) household wealth in the

Dominican Republic. Data obtained from maternal interviews on 24-hour dietary recall were extracted from the Dominican Republic Demographic and Health Survey data from 2013. Dietary consumption was classified as per the World Health Organization-Minimum Dietary Diversity Indicator (WHO-MDDI) and findings stratified by six-month age bands and household wealth quintiles (examining those currently breastfeeding and not-breastfeeding separately). Despite recommendations to avoid complementary foods in the first six months of life, substantial minorities of children less than six months of age were consuming dietary items other than breast milk or infant formula. Among non-breastfeeding 6-11 month olds, 59.7% met the recommended minimal WHO-MDDI score of four. This increased to 81.8% for those 12-17 months of age, with no subsequent consistent trend upwards with increasing age. Eggs and vitamin-A rich fruits and vegetables were the WHO-MDDI food groups, which had the most infrequent use across age groups for both those currently breastfeeding and not breastfeeding. Promotion of these food groups, particularly in the 6-11 month age period may improve dietary diversity. Unexpected was little to no difference in mean and minimal dietary diversity by wealth quintile. This may suggest nutrition education initiatives aimed at improving young child dietary diversity may not need specific tailoring by wealth strata although examination of food cost affordability by wealth strata would be important to further evaluate this recommendation.

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IS INTESTINAL PARASITE INFECTION ASSOCIATED WITH OBESITY? AN ECOLOGICAL ANALYSIS IN MEXICO

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Obesity is a worldwide healthcare challenge. Recent studies have shown an association between both viral and bacterial infection with obesity. However, studies on the association between intestinal parasites and obesity are scarce. The aim of this ecological study is to evaluate the association between the approximated probability of infection with *Ascaris lumbricoides* or intestinal protozoa (all reported intestinal protozoa excluding *Entamoeba histolytica* or *Giardia lamblia)* in 2000, 2006 and 2012 with BMI for age z-score (BMIz) in 2012 in Mexico. For this purpose, we used publicly available individual-level data for BMIz in 2012 and state-level data on the incidence of infection with *A. lumbricoides* or intestinal protozoa in 2000, 2006 and 2012 as a proxy for probability of infection. A higher approximate probability of infection with *A. lumbricoides* in 2012 was associated with a lower BMIz in 2012. In contrast a higher approximate probability of infection with intestinal protozoa in 2012 was associated with a higher BMIz in 2012. A higher approximate probability of infection with *A. lumbricoides* and intestinal protozoa in 2000 and 2006 were associated with a higher BMIz in 2012. In summary our results suggest that there may be species specific effects of intestinal parasitic infection that may have both, short and long term consequences on health. Further research is needed to confirm these ecological associations and study the possible mechanisms. These findings have important implications for Mexico, given the context of a high incidence of parasitic infection and emerging obesity epidemic.

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TEAMING UP AGAINST MALARIA: THE STORY OF SENEGAL'S SUCCESSFUL PARTNERSHIP

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Senegal's National Malaria Control Program has a long tradition of consultation with its partners. The effective partnership of government, nongovernmental organizations, donors, and communities, coordinated by strong local leadership, has enabled Senegal to achieve significant progress in the fight against malaria in the past decade. Several coordination mechanisms have helped to democratize the national response to malaria and have led the non-public sector to invest in the implementation of policies in a manner and at a level never seen before. A strong National Coordination Committee has ensured effective pooling of resources and the steady coordination of activities. In addition to activities implemented in collaboration with its traditional partners, the NMCP is also strengthening its longstanding partnership with research and training institutions. Communities have been an integral part of the NMCP's action plan since 2005. To leverage the momentum toward malaria elimination, Senegal strives to couple treatment and prevention efforts with a systematic drive to mobilize resources and catalyze the leadership of not only domestic and international governing bodies and policy-makers but communities as well to increase levels of support in the malaria fight. With campaigns such as Zero Malaria Starts With Me, the NMCP launched an inclusive national movement in favor of malaria elimination throughout communities in Senegal. Networks of community champions will be trained to raise awareness among their communities on malaria prevention and treatment and contribute to the national effort toward malaria elimination. In order for Senegal to sustain its intensive malaria control and elimination activities and achieve economic benefits, the country needs diverse and robust partnerships, both domestic and international, to ensure long-term, reliable financing for its program. Our poster presentation aims to shed light on the tremendous progress Senegal has been able to achieve thanks to strong partnerships with like-minded institutions and individuals who share the same objective: malaria elimination in Senegal.

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EVALUATION OF A MOBILE HEALTH APPROACH TO A LLIN UPTAKE INTERVENTION AMONG PREGNANT WOMEN: THE HATI SALAMA STUDY

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The Hati Salama (HASA) cluster-randomized controlled trial aims to increase malaria awareness among pregnant women using mhealth technology in Tanzania. HASA utilized an electronic system whereby nurses issue vouchers to pregnant women, allowing them to redeem a Long Lasting Insecticidal bednet (LLIN) at a retailer for a highly subsidized cost. A RCT was selected to test efficacy of SMS behaviour change communication messages aimed to increase the uptake of LLINs in areas of Tanzania identified as malaria hotspots with overall low uptake of LLINs. HASA was implemented in 97 antenatal health facilities; where 49 clinics were assigned to the control group (no targeted SMS messages sent to beneficiaries) and the other 49 in the intervention group (targeted messages sent to beneficiaries). A major study objective is evaluating the process of distributing the vouchers and understanding why they were not redeemed (approximately 30%). The investigators utilized a post-study phone survey to speak directly to those who did not redeem. The most common responses were: I went to redeem but the shop did not have any bednets; I cannot afford the co-payment; I do not live close enough to a shop that accepts the vouchers; I went to the shop but the retailer did not know how to redeem the voucher; and I did not know how to redeem the voucher. These responses demonstrate significant barriers that deter women from obtaining life-saving bednets. The process evaluation showed significant insights - the nurses revealed a large learning curve in the technology to distribute the vouchers; a trained nurse was consistently away from clinic, leaving the others overwhelmed; overall network

connectivity across Tanzania remains low, leaving women waiting for prolonged periods to receive vouchers or not at all; and many women do not have their own mobile phone. The responses collected from the participants and nurses are extremely valuable in understanding why highly subsidized bednets are not redeemed. It is imperative for donors to critically appraise these program shortcomings and barriers as many can be improved or overcome, thereby increasing bednet uptake in malaria hotspots.

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PUBLIC HEALTH INTERVENTIONS TO PROTECT AGAINST FALSIFIED MEDICINES: A SYSTEMATIC REVIEW OF INTERNATIONAL, NATIONAL AND LOCAL POLICIES

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Falsified medicines are deliberately fraudulent poor quality drugs that pose a direct risk to patient health and undermine healthcare systems, causing global morbidity and mortality. We aimed to produce a comprehensive overview of healthcare and pharmaceutical policies that could be deployed at international, national, and local scales to reduce the burden of falsified medicines in low and middle income countries (LMIC). We identified 660 studies in a systematic search of the PubMed, Web of Science, Embase, and Cochrane Library databases, of which 203 met title/abstract inclusion criteria and were categorised according to their primary policy focus: international; national; local pharmacy; internet pharmacy; and drug analysis tools. 84 were included in the qualitative synthesis, along with 108 articles and website links retrieved through secondary searches. On the international stage, we discuss the need for accessible pharmacovigilance (PV) global reporting systems, international leadership and funding incorporating multiple stakeholders (healthcare, pharmaceutical, law enforcement), and multilateral trade agreements that emphasise public health. On the national level, we explore the importance of establishing adequate medicine regulatory authorities and PV capacity, with drug certification requirements and screening along the supply chain. Local healthcare professionals can receive training on medicine quality assessments, drug registration, and pharmacological testing. Finally, we discuss novel techniques for drug analysis which allow rapid identification of fake medicines in low-resource settings. Innovative point-of-purchase systems like scratch-off mobile phone verification codes allow consumers to check the authenticity of their medicines. Such technology will be increasingly relevant to LMIC as mobile phone coverage expands, offering opportunities for integration with other "mHealth" initiatives. In summary, we describe how anti-falsifying strategies that target different levels of the pharmaceutical supply chain can be combined to protect against falsified medicines.

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CONTRIBUTION OF REMOTELY SENSED DATA FOR MALARIA RISK SURVEILLANCE IN MADAGASCAR

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In the Central Highlands (CHs), the history of malaria was marked by deadly epidemics due to the existence of unstable malaria. Disease surveillance using remote sensing becomes crucial because of environmental and climate change. It allows a better understanding of the vector behavior and disease transmission patterns. This decision tool is not yet adopted by the Malagasy National Malaria Control Program. There are no regular updates of Malagasy land cover maps and number of weather stations are not sufficient. This study aims at updating environmental and climate data. Malaria risk presents many variations at spatial scale. Second objective is to compare the relevance of information obtained from remotely sensed data with two different spatial resolutions (SR). Satellite images from Spot 5 and Landsat 8 sensors were used to classify land cover in Ankazobe district and CHs. The SR of each image was increased using pan-sharpening method. Object based image analysis method was used to classify images according to wetness, vegetation index. Climate data were acquired from NOAA and MODIS sensors. Kappa index (KI) quantifies the concordance between classification and ground truth and was used to evaluate the accuracy of the land cover classification. Two maps with five land cover classes were obtained, including rice fields, wetland, water bodies, bare soil, wood. The first three classes were highlighted because these are the preferred breeding sites of the mosquito responsible of transmission in CHs. First map is to Ankazobe district with KI of 87%. CHs are represented by the second map with a KI of 84%. The correlation between both classified images is 86%. To cover CHs, Landsat 8 needs five scenes against 40 for Spot 5 and its data processing takes less time. A 79% correlation was obtained by comparing the temperature map with the thermal band of Landsat 8 images. Landsat 8 is preferred because of its free access, satisfactory SR, wide study area and its less time consuming process. In limited resource countries like Madagascar, where national updated geographic data are rare, this allows integrating recent information in malaria risk assessment.

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DISTANCE LEARNING: REVITALIZING ANESTHESIOLOGY TRAINING IN RESOURCE-LIMITED ETHIOPIA

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Ethiopia has a significant paucity of available healthcare workers for its population of 94 million. There are an estimated 0.027 physicians per 1,000 people and specifically, 0.02 trained medical anesthetists per 100,000 people. Despite the increasing number of medical schools, there are not enough physician instructors at the schools. Furthermore, there is a lack of availability and standardization of post-graduate training. Modalities of e-learning have been shown to be successful when used to impart medical education in other resource-limited countries. The Emory University and Addis Ababa University (AAU) Departments of Anesthesiology have formed a collaboration with the intent of improving the AAU Anesthesiology Residency program, the only post-graduate training program for anesthesiology in Ethiopia. An initial educational needs assessment identified areas in the existing training program that require improvements. Interviews with faculty and residents led to study topics. In this pilot study, we describe how the current classroom-based curriculum is augmented by the introduction of interactive educational sessions and distance learning in the form of video lectures. Video lectures cover topics based on areas identified by local residents and faculty. Interactive sessions include journal clubs, problem-based learning sessions, and oral board review-type sessions. Assessment of the additions of the newly introduced blended learning technique are conducted via pre- and post-tests on the topics presented. An expansion of educational resources and modes of didactics are needed. Incorporating interactive and distance learning educational sessions into the existing didactic structure leads to improved trainee satisfaction.

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IMPROVING PREGNANCY OUTCOMES: ALLEVIATING STOCK-OUTS SITUATION OF SULFADOXINE PYRIMETHAMINE IN BUNGOMA, KENYA

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WHO recommends intermittent preventive treatment of malaria in pregnancy using sulfadoxine pyrimethamine (IPTp-SP) to be provided at antenatal care (ANC) clinic. Ministry of Health (MOH) used to procure SP until 2013 when health services were devolved to counties and procurement of became the responsibility of county governments. This presented a major challenge as counties had not factored SP in their budgets. Consequently, counties experienced SP stock-outs from October 2014. In Bungoma County the number of pregnant women receiving IPTp dropped by 51% from 7,845 in October 2014 to 3,865 in February 2015. To alleviate the situation (MOH) at national level requested counties to procure SP. Advocacy efforts with Bungoma County by the Maternal and Child Survival Program focused on prioritization of SP procurement at least once every quarter. As a result of this intervention, Bungoma County procured SP from February to July 2015. The county advised health facilities to procure additional SP doses if the supplied stocks ran out. The procurement led to a 117% increase in the number of pregnant women receiving IPTp; from 3,865 in February to 8,404 in July 2015. The fiscal year ended in June 2015 and no funds were available to procure additional SP until October 2015. This contributed to a 33% decrease in the number of pregnant women receiving IPTp from 8,404 in July to 5,672 in October 2015. As a response to support counties, MOH at national level procured 2.24 million SP doses in November/December for 14 MIP-focus counties which were received at health facilities in February 2016. In conclusion, Bungoma County applied feasible mitigation measures including county-level procurement of SP, supplemented by additional procurement at health facility and national levels. This is a practice which is replicable in other counties to ensure continued availability of SP to protect pregnant women from effects of malaria in pregnancy.

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MALARIA RISK ASSESSMENT USING MULTI-CRITERIA EVALUATION TO IDENTIFY PRIORITY AREAS FOR INDOOR RESIDUAL SPRAYING IN THE CENTRAL HIGHLANDS OF MADAGASCAR

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The malaria control strategy appropriate for the Central Highlands (CH) of Madagascar differs from the rest of the country. Indoor residual spraying (IRS) with insecticide is only implemented in the CH and Fringe regions of Madagascar due to vector behavior and efficacy of IRS. Two models of malaria risk gradient were performed in 2014 and in 2015. Malaria risk gradient was calculated for inhabited areas the CH, using Multi-Criteria Evaluation (MCE) of factors associated with malaria transmission (land cover, altitude, climate, and population data). MCE was performed by weighted linear combination method, to obtain the gradient of risk. Factor weights were determined by pair-wise comparison based on literature review and expert knowledge. In our study, fuzzy set theory was used to perform the factors' weighting. Change of risk magnitude between the two consecutive years was calculated to assess areas which shift from one risk category to another risk category within the year. The mapping of risk magnitude showed wide variation across the CH. Malaria risk gradient was categorized in five groups: very low, low, moderate, high and very high for the mapping. Risk magnitude between 2014 and 2015 showed an increase, with 1.3% and 7% risk for low risk groups and high risk groups, respectively. We observed a decrease of 1.1% for areas in the very low risk group, 2% for areas in the moderate risk group and 1.3% for areas in the very high group. However, risk status remained unchanged for 87.4% of the areas in CH. It is crucial to focus IRS efforts according to the risk gradient to improve its effectiveness, targeting only areas with the most need while optimizing available resources. Integrating data on previous intervention, malaria prevalence data from national routine surveillance or existing malaria early warning system in a Multi Criteria Decision Analysis may help improve the prioritization process for applying IRS to specific areas.

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RESISTANCESIM - DEVELOPMENT AND FEASIBILITY STUDY OF A SERIOUS GAME TO IMPROVE UNDERSTANDING OF INSECTICIDE RESISTANCE MANAGEMENT IN VECTOR CONTROL PROGRAMS

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Insect vectors transmit many human pathogens, and the cornerstone of most vector-borne disease control programs is the use of chemical insecticides. Unfortunately, the development and spread of insecticide resistance threatens the continued efficacy of these interventions, and is poised to create a public health disaster in the context of malaria control. Considerable efforts to develop new active ingredients and interventions are underway. However, it is clear that strategies to mitigate the impact of insecticide resistance must be deployed now, both to maintain the efficacy of currently available tools as well as to ensure the sustainability of new tools as they come to market. Although best practice guidelines for insecticide resistance management (IRM) have been disseminated by the World Health Organization, the lack of understanding of IRM has been identified by Rollback Malaria's Vector Control Working Group as the primary gap in the translation of evidence into policy. We developed a serious game called ResistanceSim to fill this gap. The first part of the development process convened stakeholders to define the learning objectives, target audience, and the role of mathematical models in the game. A series of learning domains were identified, and a set of specific learning objectives for each domain were defined to be communicated to vector control programme personnel. A simple "game model" was proposed as a way to produce realistic game behaviour while also capable of running in real-time. The second part of the development process was to engage software developers to map the defined learning objectives to game elements. A game design document was produced that guided the development process. An internal beta-testing phase was organized to identify any bugs in preparation for final release. The third part of the development process convened stakeholders to define the most effective strategies to roll out the tool. Finally, a feasibility study was conducted among members of the target audience in Zimbabwe. We used questionnaires and focus group discussions to evaluate users' perceptions of the tool and to identify areas of improvement.

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THE IMPACT OF LOCAL DISTRIBUTORS ON THE QUALITY OF MEDICINES IN RESOURCE-LIMITED SETTINGS: FIELD-BASED RECOMMENDATIONS

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The Sustainable Development Goal 3.8 aims at universal health coverage, including "quality and affordable essential medicines and vaccines for all". But the increasing globalization of pharmaceutical production, coupled to the lack of resources of regulatory authorities in low- and middle-income countries (LMICs), makes it difficult to thoroughly assess the quality of medicines manufactured, imported or distributed in these territories. In these contexts, the pharmaceutical distributors and wholesalers play a key-role in defining the local qualitative standards. In a previous study, we had showed the weaknesses of some international procurement agencies, in particular concerning the capacity to select pharmaceutical products based on stringent quality criteria, and to re-evaluate them regularly. As a follow-up to that study, we now looked at the compliance with WHO standards of about 30 private local distributors of medicines and medical products, located in different sub-Saharan Africa countries. The evaluation was conducted according to a set of standardised criteria, inspired by the WHO Model Quality Assurance System for Procurement Agencies (MQAS). Our preliminary findings confirm the existence of significant weaknesses, especially concerning the selection criteria for procured products and the capacity to reassess them on an ongoing basis, which means that the potential for exposure to sub-standard medicines is high. Also, clients' complaints systems may be weak or absent, which limits the possibility to identify post-marketing quality problems and to implement batch recalls if needed. To efficiently fight the plague of poor-quality medicines, there is a urgent need to improve the quality systems of private local distributors in sub-Saharan Africa. To achieve this objective, the use of harmonized evaluation tools based on the WHO Model Quality Assurance System for Procurement Agencies should be encouraged for regulatory supervision, audits and self-assessments.

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COMMUNITY EXPERIENCES WITH BEDNET DISTRIBUTION AND INDOOR RESIDUAL SPRAYING CAMPAIGNS FOR MALARIA PREVENTION IN RURAL GHANA: A QUALITATIVE INVESTIGATION

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Malaria remains one of the primary causes of morbidity and mortality in the Upper-East Region of Ghana (Ghana Statistical Service 2011). Control methods for malaria in rural areas include long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) campaigns. This research study seeks to understand the experience residents had with LLIN distribution and the IRS campaign for possible explanations of why transmission has not significantly reduced in Nangodi (World Malaria Report, 2015). Data was collected in the town of Nangodi. Researchers conducted interviews with semi-structured interview guides. Participants were sampled based on district, and included community members (46), insecticide sprayers (3), and healthcare providers (2). Participants were interviewed in Nabte and English, using a translator if needed. All interviews were recorded, transcribed, and analyzed manually for key themes. Data were cross-checked between the three authors. Information on malaria interventions was intermittent and incomplete amongst participants. Issues that came up frequently included ineffective communication of timing and instructions for spraying and worn bednets not being replaced in a timely manner. Community members who lived far from the main road reported poor bednet access and fewer visits from IRS sprayers. Community members experienced adverse events including skin irritation, stomach aches, stains on walls, and loss of fowl. In conclusion. although residents reported adverse effects from spraying, they were initially hesitant to address adverse events. Almost all would accept IRS if offered again. Residents provided important design suggestions for the campaign, such as reducing potency of the chemical and provision of face masks. Additionally, residents reported varying knowledge about the IRS campaign and many barriers to accessing new bednets. Quality and uniform distribution of information and bednets must occur in order to reduce transmission rates. Further research is needed to understand determinants of access and how best to improve current LLIN and IRS campaigns.

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PEPFAR 3.0: OPPORTUNITIES FOR ENHANCED NCD CARE WITH DIFFERENTIATED MODELS OF HIV SERVICE DELIVERY

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Since its creation more than a decade ago, PEPFAR has provided millions with life-saving ART and improved access to prevention, care, and treatment services that have drastically reduced HIV/AIDS related mortality. PLHIV can now enjoy lifespans on par with people not living with HIV. As this population ages, many also find themselves affected by a variety of non-communicable diseases such as CVD, diabetes, and a variety of cancers. Thus the treatment services provided to PLHIV could begin to change to meet these evolving needs. With the introduction of PEPFAR 3.0 and the 90-90-90 goals, HIV programs now find themselves providing even greater levels of testing, testing yield, and ART, often within the context of a flat budget. This has led to the creation of a variety of innovative solutions and approaches to both testing and treatment of PLHIV. At the forefront of these innovations are differentiated models of service delivery. Instead of offering a one size fits all approach to PLHIV care, the focus is now moving to service delivery that is tailored to the specific patient, context, and health systems within a country. As we rethink the package of services offered, and how these services are delivered to the population, there exists a great opportunity to incorporate NCD services. Multi-month prescriptions for ARVs can provide benefits to patients and providers by reducing burdens on clinics and lessening work time lost to doctor visits; however, these benefits are lessened if PLHIV continue to have monthly visits to fill their NCD medications. Multi-month prescription services require robust supply chain systems which ensure timely delivery of medications while avoiding stock outs. Looking at current developments in HIV service delivery, we identify areas and activities where the integration of NCD services would provide more holistic treatment while also working towards the 90-90-90 goals by ensuring better uptake and adherence.

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THE GLOBAL HEALTH SERVICE PARTNERSHIP: AN ACADEMIC-CLINICAL PARTNERSHIP TO BUILD CAPACITY IN NURSING AND MEDICINE IN SUB-SAHARAN AFRICA

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Thirty-one countries in sub-Saharan Africa (SSA) have a critical shortage of health care professionals, limiting healthcare delivery, response to emerging needs, and teaching and retention of future professionals. As a result, nursing and medical schools are increasing enrollment, further increasing demand on limited faculty. To address this shortage, the Global Health Service Partnership (GHSP; Seed Global Health/Peace Corps) places US nurses and physicians at institutions in SSA, working with local faculty to strengthen health education and delivery. A mixed method evaluation examined feasibility, outcomes and impact in GHSP's first 2 years (quantitative measures of productivity; qualitative data from stakeholder interviews). Between July 2013-June 2015, 69 GHSP educators were deployed to 13 universities in Malawi, Tanzania, and Uganda. They provided 85,612 service-hours, taught 300 courses to 7,219 trainees, participated in curricula revision and development (including new post-graduate MSN and MMed programs), enhanced the teaching infrastructure, and made academic-clinical linkages to facilitate practice improvement projects. Qualitative data revealed the largest impact on students (the provision of quality, consistent education, particularly clinical supervision; value added to the learning environment; and increased student confidence and empowerment). Impact on faculty was significant in the areas of workload reduction, mentoring, and value added to the learning environment. Taken together, these data suggest that an innovative, locally tailored and culturally appropriate academic partnership is feasible and generated new knowledge and best practices relevant to capacity strengthening for nursing and medical education. Key features of GHSP include the intentional pairing of US/African educators, emphasis on faculty supervised clinical instruction, and a sustained commitment over time. Continued evaluation will inform optimizing classroom and clinical pedagogy in resource-constrained settings and improve the health and wellbeing of populations who suffer a high burden of disease.

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ANTIMICROBIAL ACTIVITIES OF SIX SELECTED MEDICINAL PLANTS AGAINST *STAPHYLOCOCCUS AUREUS*

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The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. For a long period of time, plants have been a valuable source of natural products for maintaining human health. Therefore, such plants should be investigated to better understand their antimicrobial properties, safety and efficacy. The study evaluated *in vitro* antimicrobial activities of aqueous and ethanol fractions of six selected medicinal plants, including *Eugenia cryophyllata*, *Psidium guajava*, *Alchornea cordifolia*, *Cinnamomum zeylanicum*, *Zanthoxylum xanthoxyloides* (*fagara*), and *Tridax procumbens* against 19 clinical isolates and a standard strain of *Staphylococcus aureus* using a modification of the agar diffusion method. The potency of 16 formulations from five of the medicinal plants that showed significant antimicrobial activity was also evaluated. The ethanol fractions inhibited the growth of the test organisms with zones of inhibition ranging from 4.0-20.5 mm averaging 8.6 mm whereas that of the aqueous fractions ranged from 4.0-20.5 mm averaging 7.4 mm. Formulations from the combinations of *Alchornea cordifolia* and *Eugenia cryophyllata* showed inhibition zones ranging from 9.0-16mm and 7-16 for the aqueous and ethanol extracts respectively. There was significant difference between the ethanolic and aqueous plant extracts against *S. aureus* used in this study. Minimum inhibition concentrations (MICs) of the herbal preparations used against the control strain (*S. aureus* ATCC® 29213™) and the clinical *S. aureus* isolates showed that, both the aqueous and ethanolic extracts of *Alchornea cordifolia* and ethanol extract of *Cinnamomum zeylanicum* produced the lowest MICs of 2 mg/ml. Considering the fact that these extracts are crude, it could be inferred that they possess antibacterial activities that need further investigations to identify the compound(s) responsible for the antibacterial activities.

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CREATING A HOLISTIC FRAMEWORK FOR UNDERSTANDING THE FULL IMPACT OF THE ZIKA VIRUS ON MOTHERS, INFANTS, HEALTH SYSTEMS AND SOCIETY

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In February of 2016, the World Health Organization (WHO) declared Zika Virus as a Public Health Emergency of International Concern under the International Health Regulations. Zika is projected to take a huge toll on the health and wellbeing of people across the Americas as well as globally, given international climactic and transport pathways for dispersion. Zika now presents a new challenge, with implications beyond generalized mild infections, suggesting considerable risk in pregnancy of a clear malformation in the child and longer-term effects on proper brain development and this will have important effects on costs of health and social care. The most likely impact on the majority of households is that someone, usually the mother, will become a long-term caregiver, often taking women out of the workforce and creating extra strain on household finances leading to a greater risk for financial shocks that tend to lead to poverty. In many low- and middle-income countries, the increase in women in the workforce has been a stabilizing factor in household welfare, pulling many households out of poverty. Mothers forced to become full-time caretakers could see that progress reversed. Alternatively the threat of having a malformed child can lead the mother to search for a way to terminate her pregnancy. In countries with strong anti-abortion policies, unsafe abortions could negatively affect maternal mortality. If

a period of high fertility is followed by a sudden period of low fertility, after which the rate picks up again, there will be a risk for the balance of dependents in future generations. Tax and public service provision systems rely on there being a good match between working age populations and dependents, such as the young and the retired. Using widely available data sets containing data both fertility rates, formal and digital incidence trends of Zika, unsafe abortions, health care utilization, female workforce participation and income and poverty levels we developed a simple epidemiological-economic model to estimate the likely long term impact on the health, health care costs social welfare and poverty of households across the Americas.

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USING AMR DATA IN DATA-RICH COUNTRIES IN COMBINATION WITH UNIVERSAL 'MEDIA' DATA TO MAP ANTIMICROBIAL TRENDS AND DRIVERS ACROSS DATA-POOR AREAS OF AFRICA

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The World Health Organization has highlighted antimicrobial resistance (AMR) as a "major global threat" to public health. Resistance is growing in every region of the world, including resistance to 'last resort' antibiotics. In the United States, an estimated two million illnesses and 23,000 deaths are attributed to antibiotic resistant bacteria or fungi each year. The key drivers of AMR are largely known; antimicrobial overuse or misuse, suboptimal dosing, including from substandard and falsified drugs, suboptimal diagnostics and vaccination. Despite this knowledge, the complexity of the interaction between these factors and how each affects the scale of the problem in different contexts is heavily reliant on the health care system, rules around prescription and use of antibiotics, and the prevalence, mix and concentration of disease burden from bacterial diseases in each country. In addition to this context specific complexity, the countries that suffer most from bacterial infections are often those that are most data-poor in terms of both routine laboratory testing of patients and systematic data collation on the existence and trends of AMR drivers. To better assess the risk of AMR across countries where the impact could be most devastating requires a more novel approach to generating real time estimates of both the prevalence and rate of growth of AMR. We have developed a data collection and analytic framework that utilizes three distinct sources of routine data, but which mixes data from data-rich settings and data-poor settings, using a method of triangulation to extrapolate gaps in prevalence and growth of AMR in data-poor countries. These data sources are: 1) data on annual incidence of AMR in data rich countries from official government surveillance reports; 2) digital real-time reports acquired from HealthMap Resistance Open, which aggregates online news, social media and hospital reports of AMR events; and 3) data on key AMR drivers from routine healthcare and pharmacy data, and local drug policy and regulation data. We show how this framework could be used to monitor real time trends in AMR and its relationships to key drivers of AMR.

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ZIKA VIRUS-RELATED PHOTO SHARING ON PINTEREST AND INSTAGRAM

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Communicating accurate, accessible and actionable information to diverse populations is a key component of emergency responses against the Zika virus outbreak. Public health agencies are engaging the public using fast-growing photo-sharing social media sites such as Pinterest and Instagram. In this cross-sectional study, 616 Pinterest photos (keyword: "zika" AND "virus"; the maximum number of photos that we were able to retrieve via web scraping) and 616 Instagram photos (#zikavirus; randomly selected from 9370 Instagram photos retrieved via Instagram Application Programming Interface) were retrieved on April 3, 2016. Two trained individuals manually coded photos based on their relevance to Zika virus, words embedded, language and their content categories (any category that applies). Among our samples, 47% (290/616) of Pinterest photos and 23% (144/616) of Instagram photos were relevant to Zika virus. Words were embedded in 57% (164/290) of relevant Pinterest photos and 100% (144/144) of relevant Instagram photos. Among the photos with embedded words, more Instagram photos were in Spanish and Portuguese (77/144, 53%) than Pinterest (14/164, 9%) ($P<0.0001$). There were more Zika virus-related photos on Instagram than on Pinterest containing prevention information (59/144, 41%, versus 41/290, 14%, $P<0.0001$), issues relevant to effects on pregnancy (27/144, 19%, versus 32/290, 11%, $P=0.04$) and deaths associated with this infection (4/144, 2%, versus 0/290, 0%, $P=0.01$). Given the relatively international demographics of Instagram (vis-à-vis Pinterest that is more American), it is reasonable to suggest that Latin American users were more concerned about Zika virus prevention and its impact on pregnancy outcomes. In conclusion, Pinterest and Instagram have similar representation of Zika virus-related photos. Health communicators may use both Pinterest and Instagram as similar platforms to disseminate Zika virus information to the public.

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IMPROVEMENT OF CHILD NUTRITIONAL STATUS IN THE DEMOCRATIC REPUBLIC OF CONGO OVER TIME: SERIAL CROSS-SECTIONAL ANALYSIS OF THE DEMOGRAPHIC AND HEALTH SURVEY

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In the Democratic Republic of Congo (DRC), prolonged conflict, high levels of infectious disease and severe poverty take a toll on the health and development of children. Approximately one in seven children die before reaching the age of five and approximately half of these deaths are due to malnutrition and nutrient deficiencies. To assess the change in child health status over time, we utilized nationally representative, cross-sectional data from the 2007-2008 and 2013-2014 DRC-Demographic and Health Surveys. Data from those under 5 years of age included but was not limited to height, weight, anemia and health outcomes among 3,951 children in the first and 8,552 children in the second wave of the survey. According to child anthropometric measures, a significant improvement ($p<0.0001$) was observed between 2007 and 2013 for stunting (height-for-age, 55% and 46%), wasting (weight-for-height, 27% and 13%) and underweight (weight-for-age, 38% and 27%). Such improvements varied

by province (percent change range: stunting, 4% to 45%; wasting, -12% to 69%; underweight, -13% to 68%) and place of residence (with the greatest improvements observed in urban compared to rural settings). Additionally, while significant improvements (p<0.0001) were observed for anemia between 2007 and 2013 (hemoglobin count <11 g/dl, 71% and 50%, respectively), little change was observed in moderate to severe estimates during this time (hemoglobin count <10 g/dl, 2007: 23%, 2013: 21%). While location of residence (urban/rural) was not associated with anemia, estimates did vary by province (percent change range: 15% to 53%). As poor growth performance during infancy and early childhood is commonplace in many developing countries, it is important to track how these measures change over time. Malnutrition is a major contributor to impaired intellect, increased mortality and susceptibility to infection, thus such surveillance allows for the identification of vulnerable subgroups to whom intervention efforts may be targeted to improve the health and development of children.

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CAN WE TARGET MOBILE POPULATIONS WITH AN INTEGRATED DISEASE CONTROL APPROACH? A QUALITATIVE STUDY ON RISK BEHAVIOR FOR MALARIA AND HIV ALONG THE THAI-CAMBODIA BORDER

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The border zone in Oddor Meanchey province in Cambodia is characterized by high human mobility, as economic opportunities such as logging, farming and plantations attract migrants. Malaria parasite resistance has been detected in this region, requiring active case detection and management. High prevalence rates for HIV also occur among high-risk groups such as female entertainment workers, active around the border casinos and catering to loggers and the military. This qualitative study, carried out in collaboration with the national control centers of Malaria (CNM) and HIV (NCHADS), aimed to characterize the vulnerabilities of different types of mobile groups in order to propose integrated control activities. Between December 2014 and 2015, semi-structured and open-ended interviews (N=195) were conducted in Oddor Meancheay with theoretically and snowball-sampled informants. Four main categories of mobile populations were identified whose activities exposed them to malaria and HIV: (i) military men, (ii) local farmers, (iii) rural migrants working on plantations, (iv) female entertainment workers. The first three categories of people support their families by forest and farm work, which increases the risk of malaria since during nightly logging or overnight farm stays people lack malaria preventive measures. Additionally, income produced by these activities is partly spent in entertainment places, both in temporary camps in the forest and around casinos along the border, where alcohol consumption and drug use lead to unsafe sexual practices. Tests and treatment for HIV and malaria from the public sector or the Village Malaria Worker are less popular than those bought in the private sector, where privacy and quality are expected. As such, many HIV and malaria cases go undetected or are not followed up by adequate treatment. As certain risk behaviors tie these mobile populations together, integrated HIV and malaria control activities are feasible, potentially through the active detection of at-risk individuals by both HIV and malaria programs in collaboration with the military and private medical sectors.

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BANGLADESH OBSERVED NATIONWIDE MEASLES-RUBELLA VACCINATION CAMPAIGN

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Bangladesh observed nationwide Measles-Rubella (MR) vaccination campaign from 25 January to 13 February 2014. This three weeks rolling campaign supplemented and complemented the routine immunization effort to achieve and maintain high population immunity against measles and rubella as well as to provide a second opportunity for measles-rubella (MR) vaccination among the susceptible children. About 52 million children aged 9 months- <15 years were targeted for MR vaccination through this campaign. The key objective of the MR campaign was to achieve at least 95% coverage of measles and rubella to develop high population immunity against these two diseases. 52,745,231 children aged 9 months to <15 years were enlisted to vaccinate with MR vaccine through educational institutions and community EPI sites. School going children were vaccinated in educational institutions and non-school going children were vaccinated in community sites. There were about 157,983 community sites and 158,555 educational institution sites to give MR vaccine. For successful outcome, efforts were given on advocacy and communication. All vaccinators received training on MR vaccination and campaign activities. Orientation programs were organized for volunteers and school teachers in view to seek their cooperation during campaign. The national EPI team and partner organizations observed that the presented administrative coverage data were good at national level and for most of the districts and city corporations. As per micro-plan 53,644,603 (101.7%) had been vaccinated, among them 32,933,783 (62.4%) children were vaccinated in educational institutions and 20,710,820 (39.3%) children were vaccinated in community sites. MR campaign was conducted safely and minimum number of AEFI happened and reported.

829

MOTHER AND CHILD PAIRS DOUBLE BURDEN OF MALNUTRITION IN THE SAME HOUSEHOLD, IN BENGO PROVINCE, ANGOLA

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The coexistence of undernutrition and over nutrition, known as double burden of malnutrition, presents significant threats to human health, especially in countries that are undertaking rapid economic development. In this countries, factors as the diet transition from high carbohydrates, low fat diet to a diet with refined grains, meat, oils and dairy products, all high fat content is happening. The present study, included in a cross sectional study in Bengo Province Angola, aims to identify modifiable factors associated with the occurrence of mother overweight and child underweight in the same household. A total of 622 mother child pairs were included in the analysis. From the total of 622 mothers, 6.4% were underweight (BMI < 18.5), 60.3% were normal weight and 23,3% were overweight (BMI 25-30) and 10% were obese (BMI >30). In what concerns the children, wasting was observed in 20,1 % (WHZ score under -1), stunting in 61.9% (HAZ score under -1) and underweight in 42,9 % (WAZ score under -1). Concerning mother/child pairs, 11,1 % of the pairs revels a relation of overweight mother/underweight child, 32,2% normal weight pair, 22.2% overweight mother /normal weight child, 28.1% normal weight mother /underweight child and 3.7% were both underweight. The pairs overweight mother / underweight child (n=200), and normal weight mother and child (n=69) were included in a case control analysis. Overweight mothers tend to be older, and with lower frequencies attending secondary or university studies. The study did not report any association between dual burden of malnutrition and mother employee, age and gender of the child, household size or Household month income. A significant association was observed in the multivariate analysis with place of birth, with an apparent risk reduction with home born. The

present study confirms the existence of dual forms of malnutrition in the same household in Angola. Further studies are necessary to understand the factors responsible for this coexistence. Moreover, this information is vital in the preparation of food intervention programs addressed to the adoption of proper and healthy behaviors.

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JOINING THE HALVES: A PRIVATE PUBLIC PARTNERSHIP TO MAKE ROUTINE HEALTH REPORTING ATTRACTIVE TO PRIVATE SECTOR PROVIDERS IN UGANDA

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An estimated 50% of fever cases seek care from private-for-profit (PfPs) providers, highlighting their significant role in serving the population in Uganda. Although all PfPs are mandated to report health data, there are challenges with non-availability of private sector friendly tools and this has hindered regular reporting of health data by PfPs, resulting in limited knowledge of the private sector contribution to disease control and surveillance. As part of the UNITAID-funded project to expand malaria rapid diagnostic tests (mRDTs) use in Wakiso, Uganda, 289 participating outlets were trained and availed with a user friendly individual-level data register to strengthen routine monitoring. The register was developed in collaboration with the District Health Team (DHT) and was compatible with the Health Management Information System (HMIS). Reporting outlets were incentivized by awarding certificates of recognition during district-led provider meetings. The creation of this tool, improved capacity for reporting on HMIS malaria indicators was achieved, with 10 (3.5%) outlets reporting at baseline in August 2014 and 210 (72.7%) outlets reporting in June 2015. The collaborative involvement fostered a partnership between the PfPs and the DHT and provided a platform for PfPs to transition to directly report into the HMIS. Customizing data collection tools to the private sector, administering trainings that highlight the benefits of reporting, promoting public-private partnerships and provision of non-monetary incentives has demonstrated improvement in compliance of reporting essential routine health indicators. Strategies for expansion will be presented.

831

IMPACT OF MEASLES MASS-VACCINATION CAMPAIGN AMONG THE CHILDREN UNDER FIVE YEARS OLD IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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Measles is a highly contagious viral infection. Initial signs and symptoms typically include fever >40°C, rash, cough, runny nose, and inflamed eyes. Measles continues to be a leading cause of vaccine-preventable deaths worldwide, most of which occur in resource-limited countries. In 2014, an estimated 17,574 measles cases were reported in the African region. In the Democratic Republic of Congo (DRC) epidemics of measles have been reported since 2010 due to postponed supplementary immunization activities (SIAs). Since, provincial level SIAs have been conducted in each province.Using dried blood spots collected during the 2013 Demographics and Health Survey (DHS), we assessed the seroprevalence of anti-measles IgG among children children 6-59 months of age in the DRC. We overlaid SIA data obtained from DRC's Expanded Programme on Immunization (EPI) to determine if there were correlations between vaccination campaigns and measles immunity, and calculated the Correlation Coefficient.
In provinces that experienced an SIA in 2013, 77% of children were seropositive for measles, while in provinces with no SIA, only 59% of children were seropositive. The correlation coefficient is 0.420.Our findings suggest the importance of a strong routine immunization program coupled with frequent SIAs. While we were unable to determine whether Repeated occurrences of large-scale outbreaks in DRC suggest the need to reevaluate and modify DRC's measles prevention and control strategies to meet regional elimination goals.

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SEVERE MATERNAL MORBIDITY AND ASSOCIATED FACTORS IN TWO LARGE HOSPITALS IN THE ASHANTI REGION, GHANA

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Ghana's maternal mortality ratio is one of the highest in the world. To achieve significant reduction in maternal mortality there is a need to go beyond analyzing mortality and explore the risk factors of maternal morbidity. Studying Severe Maternal Morbidity (SMM) enables faster quantitative analysis and makes it possible to obtain in-depth information on the affected woman herself. The research was therefore conducted to determine factors contributing to severe maternal morbidity in Suntreso and Kumasi South Government hospitals in the Ashanti region of Ghana. A case control study was conducted at the Suntreso and Kumasi South Government Hospitals of Ghana between January 2015 and June 2015. WHO near miss classification system was used to identify maternal near-miss. Univariate analyses of categorical variables were expressed as frequencies and proportions. Factors independently associated with severe maternal morbidity were determined by multivariate analysis with a significance level of 5%. Among 2,238 pregnant women, 15 maternal near miss (MNM), 7 maternal deaths (MD) and 71 potentially life-threatening conditions (PTLC) were identified. The maternal mortality ratio was 229.6 cases/ 100,000 LB with a mortality index of 31.8%. The most diagnosed potentially life threatening condition was postpartum hemorrhage (57.7%). Risk factors of severe maternal morbidity identified were preterm delivery (<37 weeks) [aOR 7.8 95%CI (3.0 - 20.2)], caesarean section in current pregnancy [aOR 9.7 95% CI (3.1-30.2)], and anemia during the current pregnancy [aOR 8.1 95% CI (2.9 - 22.2)]. Factors associated with SMM were preterm delivery, caesarean section in current delivery and anemia in current pregnancy. The use of herbal preparation during pregnancy though not associated with SMM was used by almost half of the study participants.

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FACTORS AFFECTING REPORTING RATES OF COMMUNITY VOLUNTEERS IN AN MHEALTH INTERVENTION REPORTING DATA THROUGH CELL PHONES

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In lower-income countries, many mHealth solutions have been devised to empower health workers and facilitate information systems. For example, in Zambia, cellular phones are used to transmit information on malaria trends as well as water and sanitation access using community volunteers. mHealth solutions, and particularly those using volunteers, are attractive to global health programs for a number of reasons including the rapid transmission and availability of data as well as the cost-efficiency of using community volunteers to generate and send data. The strength of these mHealth information systems lies in the reporting rates of the data and the opportunity to gather data from community-level. Little research however is available on what influences reporting rates. From our experience in mHealth we have seen systems with <15% reporting each time period,

but also other systems with >85% reporting each time period. We utilize information system data from the WASH information system in Zambia to determine factors associated with reporting rates first at the district level, and then by community volunteer. We measure the impact of seasonality, remoteness (an indicator of cell coverage), increasing the number of data elements reported and follow-up from national-level staff. These analyses are planned for April 2016, with results available by May 2016. In this presentation we will discuss what factors influence reporting rates for mHealth surveillance solutions and the implications for global health programs utilizing mHealth.

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IMPACT OF COMPUTERIZED PHYSICIAN ORDER ENTRY (CPOE) SYSTEM IN PREVENTING PRESCRIPTION ERRORS IN ADMITTED PEDIATRIC PATIENTS IN A DEVELOPING COUNTRY

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Prescription errors could account for up to three-quarters of iatrogenic deaths especially in the pediatric settings. Computerized Physician Order Entry (CPOE) with decision-support analysis has shown to be effective in reducing errors in resourceful settings. In resource-limited setting, the implementation of CPOE is challenging. This study evaluates the impact of CPOE in preventing prescription errors in a low-income setting.In this quasi-experimental study, patients admitted to the pediatric department Mayo Hospital Lahore, Pakistan were studied. The main intervention was a CPOE system with decision support analysis that was installed and piloted over the period from January to May 2014. Prescriptions of patients admitted in the period from seven months before the implementation and after the implementation were assessed by a resident. Physician only prescriptions continued to be issued in the post-implementation period and were included in the comparison. The final analysis compared the physician errors per 100 patient days between the physician only and the CPOE system.The sample included 2156 patients, 1103 in the pre-implementation phase and 1053 in the post-implementation period. The error rate for physician only prescriptions was 29.4 per 100 patient days. The error rate for CPOE system (3.9 per 100 patient-days) were nearly seven times less than the physician only system (Incidence Rate Ratio = 0.13; 95% Confidence Interval = 0.11 - 0.13). The error rate in physician only prescriptions was almost two times higher for patients who died than those who were discharged (47.4 vs. 23.5 per 100 patient-days). The error rates were also higher in patients in the CPOE group who died compared to those discharged (6.0 vs. 3.5 per 100 patient-days).CPOE system is feasible and effective in a resource-limited pediatric setting. Healthcare stakeholders in the resource-limited settings might need to prioritize CPOE like systems to prevent iatrogenic deaths.

835

LINKING HOUSEHOLD AND POINT-OF-CARE DATA TO ESTIMATE COVERAGE OF APPROPRIATE MANAGEMENT OF CHILDHOOD ILLNESS IN SOUTHERN PROVINCE, ZAMBIA

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Studies of indicator validity have demonstrated that household surveys may inaccurately estimate coverage of health interventions, including treatment of key child illnesses. Where household surveys are insufficient to estimate coverage, there is need for new methods for generating accurate measures of health intervention coverage for measuring global progress. Linking population-based data with point-of-care (POC) information on service readiness and quality of care (QOC) has been proposed as a means of generating more accurate coverage estimates. A 2016 survey was prospectively designed to collect temporally and geographically proximate population-based care-seeking data and POC readiness / QOC data to estimate coverage of appropriate management of child illness. Mothers of children <5 years old in Southern Province, Zambia were randomly sampled. Reported care-seeking events were ascertained in each household using a questionnaire modeled off the Zambia Demographic and Health Survey. Information on service readiness and QOC for child curative services was collected within 6 weeks of the household survey from all significant POCs in the study area, including public, private, informal, and traditional providers. Service readiness was assessed using a survey tool modeled off the WHO service availability and readiness assessment (SARA). Care-seeking data were collected for 537 children in urban areas and 547 children in rural areas. Service readiness and QOC data were collected for 75 POCs. Household and POC data were combined using an exact-match linking method, which assigned each illness episode a value for likelihood of appropriate treatment based on the POC readiness / QOC score of the reported source(s) of care. These values were then used to generate coverage measures for appropriate management of child illness. Analogous coverage measures were generated using 4 common geographic linking methods, and compared against the estimates generated through the exact-match method to assess bias introduced through geographical linking methods. Implications and recommendations for coverage measurement will be discussed.

836

USING THE KEMRI-CDC HEALTH AND DEMOGRAPHIC SURVEILLANCE SYSTEM TO DEMONSTRATE THE CHANGING NEONATAL MORTALITY RATE BETWEEN 2003 AND 2012 IN RURAL WESTERN KENYA

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Understanding the burden and causes of neonatal mortality is necessary for making progress towards Millennium Development Goal #4. We examined the overall rate, trends, epidemiology, and factors associated with neonatal mortality in the Western Kenya Health and Demographic Surveillance System (KHDSS), 2003 to 2012. The KHDSS, located in Bondo and Siaya Counties in rural Western Kenya, covers an area of 371 sq. km and a population of 159,000 people. Village reporters notify about all births and deaths. Standardized verbal autopsy questionnaires were completed by trained interviewers and the cause of death was assigned by a panel of three clinical officers, using ICD10 codes. Neonatal (≤28 days) and early neonatal (≤7 days) mortality rates were calculated per 1,000 live births. We explored factors associated with neonatal death using logistic regression to compare deaths to non-deaths among neonates. During 2003-2012, there were 52,385 live births, 899 neonatal deaths and 634 (70.5%) early neonatal deaths. The overall neonatal mortality rate (NMR) was 20.1 per 1000 live births (95% confidence intervals [CI] 19.7 -25.2) and early NMR was 14.4 per 1000 LB (95%CI 11.5-18.2). Neonatal deaths represented 18.3% of infant deaths. Higher maternal age, secondary education, and higher SES were significantly less common among neonates that died compared with those that survived. Verbal autopsies were done for 533 (71.3%) of neonatal deaths. Overall, the leading causes of death were neonatal sepsis (39%), pre-maturity (18%), and respiratory distress (10%). The leading causes of death in the first week of life were neonatal sepsis (28.8%) and pre-maturity (18.6%). Medical care was sought prior to death for 106 (11.8 %) neonates overall, including 45 (7.1%) of those that died in the first week of life. The burden of neonatal

mortality in Western Kenya is high, and the majority of neonatal deaths occur in the first week of life. Most neonates die without having received medical attention for their illness. Deaths from neonatal sepsis might be reduced through community-based management to ensure earlier access to antibiotic treatment.

837

STUDENTS AS AGENTS OF CHANGE: EXPERIENCES FROM SUDAN IN UPDATING NATIONAL SCHOOL CURRICULA TO INCLUDE TRACHOMA MESSAGING

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Beginning in 2014, the Sudan national trachoma control program (NTCP) began developing methods to incorporate trachoma messaging into their respective national and regional school sanitation guidelines. The aim was to focus attention on school-based programming that would result in schools and students becoming more involved in sanitation campaigns in the community thereby becoming agents of change within their homes and communities. In the Republic of Sudan, the NTCP has worked to engage the Federal Ministry of Health (FMOH) and the Federal Ministry of Education (FMOE) in an effort to coordinate the development of school health education materials. As part of this process, The Carter Center and NTCP developed school health education materials, while the National Centre for Curriculum and Education Research and FMOE revised and approved the trachoma curricula. Together, the FMOH, FMOE, NTCP and The Carter Center produced teachers' guidelines for basic and secondary schools on how to deliver information related to trachoma control. In 2015, 72 state coordinators, education inspectors and school hygiene coordinators were trained on how to be trainers of others. An additional 2,000 teachers were trained on the trachoma curricula and over 105,000 trachoma curricula and 1,900 manuals have been distributed in basic and secondary schools. Though the implementation of these activities are currently limited to the areas in which The Carter Center is assisting the FMOH and NTCP, it is hoped that they will provide a model that can be expanded to other parts of the country and used as an example for neighboring endemic countries that seek to integrate trachoma messaging into their own curriculum.

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GENERATING AN ELECTRICITY ACCESS MAP ACROSS AFRICA

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For many health outcomes, there is a strong relationship with socioeconomic status. Demographic and Health Surveys (DHS) collect information about household indicators related to wealth in different countries across time. One such indicator is access to electricity. Unfortunately, due to the varying periodicity and spatial coverage of these surveys, the information they contain is not representative at a regional scale. Fortunately, unlike other indicators such as access to drinking water or education, access to electricity can be indirectly measured via remote sensing. National Oceanic and Atmospheric Administration satellites have been capturing images of nighttime light globally since 1992. While nighttime light images do not immediately translate into electricity access of households, we define a model to learn the association between both. We use information from 73 DHS surveys, light intensity extracted from inter-calibrated night light images and population density. We follow a Bayesian model based geostatistics approach, and use integrated nested Laplace approximation (INLA) to make inference on household's electricity access in Africa. Results show that access to electricity has a positive log-linear relationship with nighttime light intensity and population density. The model is used to predict the probability of a household having electricity annually from 2000-2015 across Africa at 5km resolution. These freely available maps describe the story of electrification across Africa and will be provide insight, in future work, on the link between infectious diseases and socioeconomic resources in the region.

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VOTING FOR BETTER HEALTH IN DEPRIVED COMMUNITIES

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The Global Health Exchange Fellowship was a pilot project, designed to make global health real through experiential learning for UK and Kenyan trainees in family medicine/general practice and public health.Using Qualitative research methods, a health needs analysis was performed in two deprived areas- a rural Maasai community in Kenya and an inner city in the UK. Health issues identified were categorised into themes, which were prioritised by the community using an innovative voting methodology developed by the fellows. The same methods were applied in both countries.The voting method allowed each community a voice, in prioritising their health needs. Using the Capability Approach sustainable solutions were sourced within the community. Findings were presented to local health authorities to inform local resource allocation, improve health and reduce inequalities.The fellows learned a great deal about global health challenges in both high and low income countries. A methodology of community voting was established, providing insight to the true health needs of each community.This methodology provides new understanding from the perspective of two communities on global health, including social determinants of health. There is remarkable potential for its widespread use. Similarities in themes in areas of deprivation in low and high income countries is noteworthy. In Kenya, access to healthcare was voted as the number one priority. Had we taken an epidemiological approach, we may have found ourselves tackling specific diseases. However, the voting method identified the health issues that were much closer to the true health needs of the community. This was particularly important in Kenya, where there was no data available for our community. In the UK there is a wealth of data, therefore this project sought to address the "Why" and the "How", thus developing sustainable strategies to address health needs, whilst encouraging community ownership through Sen's Capability Approach.

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A MULTI-'OMIC SYSTEMS BIOLOGY APPROACH TO IDENTIFYING HOST AND PARASITE FEATURES THAT CONFER RESILIENCE TO MALARIA INFECTION

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The Malaria Host-Pathogen Interaction Center (MaHPIC) and the Host Acute Models of Malaria to study Experimental Resilience (HAMMER) Project are intertwined systems biology investigations that are generating a wide array of biological, clinical and multi-omic data sets of diverse *Plasmodium* species in their non-human primate (NHP) and human hosts. Using NHP models, the MaHPIC project is investigating changes in host

immune status, erythrocyte phenotype, and metabolic state alongside changes in parasite abundance, stage, and gene expression through the use of clinical and multi-omic measurements. Using both mathematical modeling and data integration approaches, we are identifying multi'-omic profiles that are associated with the observed variation in the severity of malarial disease in the animal cohort studies. These studies involve *Plasmodium cynomolgi* and *P. coatneyi* infections of *Macaca mulatta* (rhesus monkey) to model *P. vivax* and *P. falciparum*, respectively; as well as *P. vivax* infections of the New World monkey species *Aotus nancymaae* and *Saimiri boliviensis.* Further, we are conducting high-resolution metabolomics analyses in plasma from human clinical cohort studies led from South America, Southeast Asia and Sub-Saharan Africa. Upcoming work will focus on characterizing multi-omic profiles of infection by *P. knowlesi* in two different host species with differing susceptibility to malaria disease, *M. mulatta* and *M. fascicularis*. The aim of this work is to identify host features that confer resilience to malarial disease. Altogether, we aim to identify novel host and parasite factors involved in malaria disease progression in NHPs, and translate these findings to what is observed in humans. The MaHPIC and HAMMER projects include an extensive bioinformatics infrastructure to generate and release datasets for use by the broad scientific community.

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MOLECULAR DISSECTION OF THE *PLASMODIUM* SPOROZOITE SURFACE GAPDH FOR MALARIA LIVER INVASION

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Malaria, caused by parasites of the genus *Plasmodium*, is among the most devastating parasitic diseases worldwide. The bite of an infected *Anopheles* mosquito releases less than 100 sporozoites in the skin but after liver infection up to 10,000 merozoites per sporozoite are produced and released into the circulation. Therefore, the pre-liver hepatic stages represent a severe bottleneck in parasite numbers and constitute a prime target for induction of sterile immunity. To infect the mammalian host, parasites must leave the circulation in the liver by preferentially traversing Kupffer cells that together with endothelial cells, line the liver blood vessels (sinusoids). Previously we have identified CD68 on the Kupffer cell surface as a receptor for sporozoite traversal. We now report that *Plasmodium* GAPDH on the sporozoite surface serves as a ligand that interacts with CD68. Current experiments seek to define GAPDH domains involved in this interaction. Such domains are potential epitopes for the development of a pre-erythrocytic vaccine.

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PFEBA-175 MEDIATED ROSETTING ENHANCES GROWTH OF *PLASMODIUM FALCIPARUM* AND OVERCOMES INHIBITORY ANTIBODIES: IMPLICATIONS FOR SEVERE MALARIA

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Severe malaria is defined by high parasetemia and anemia and results in the majority of fatalities due to *Plasmodium falciparum* infection. The mechanisms that enable high parasite burdens in patients resulting in severe malaria and death are poorly defined. Here, we show that PfEBA-175 shed from parasites during invasion to form infected RBCs (iRBCS) facilitates the recruitment of uninfected RBCS (uRBCS) to form rosettes. This recruitment increases parasite growth resulting in a defined fitness advantage that enables high parasitemia observed in severe malaria. This is the first demonstration of growth enhancement directly due to rosetting. We show that rosette formation is dependent on PfEBA-175 engagement of the receptor Glycophorin A and identifies a novel role for the PfEBA-175:Glycophorin A interaction in addition to RBC invasion. We propose that rosetting allows for invasion of adjacent uRBCs from one iRBC, bypassing the need for daughter merozoites to search and identify uRBCs to invade in the bloodstream. This may be one mechanism by which parasites achieve the high parasite burden seen in severe malaria. Rosetting also reduces the time in which the merozoite can be targeted by the immune system resulting in immune evasion. Further, we show that rosetting overcomes the effectiveness of antibodies known to inhibit growth. The results emphasizes the need to include PfEBA-175 in anti-malarial combination therapies that aim to block rosette formation and enable the function of neutralizing antibodies. Lastly, these data demonstrate that shed proteins may confer additional functions to enhance parasite survival and opens new avenues of research for severe malaria.

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PLASMODIUM VIVAX INFECTIONS AMONGST DUFFY-NEGATIVE INDIVIDUALS IN THE DEMOCRATIC REPUBLIC OF THE CONGO: POSSIBLE ACQUISITION FROM APES

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The World Health Organization estimates that 10% of global malaria cases and deaths occur in the Democratic Republic of the Congo (DRC) annually. *Plasmodium falciparum*, *P. ovale*, and *P. malariae* account for the great majority of cases. However, little is known about *P. vivax* infection in the DRC. We selected 618 dried blood spots in the 2013-2014 Demographic and Health Survey of the DRC, a large population-based survey. Four cases of *P. vivax* infections were identified by PCR, each in a geographically different survey cluster. Using these as index cases, we tested all the samples from the four clusters. With this approach, an additional ten cases of *P. vivax* were identified. Among the fourteen *P. vivax* cases, nine were coinfected with *P. falciparum*. To assess host susceptibility to *P. vivax*, we PCR-amplified and sequenced each host's Duffy antigen/chemokine receptor gene (DARC) for the single point mutation in the GATA motif that represses the expression of the Duffy antigen. All fourteen hosts infected by *P. vivax* were Duffy-negative. This finding is consistent with a growing body of literature that suggests that *P. vivax* can infect Duffy-negative individuals in Africa. Next-generation sequencing of the mitochondria of four of these infections suggests that at least one of these infections contains strains of both human and ape origin. Currently, we are exploring the origins of these *P. vivax* infections using phylogenetically informative regions on six loci that have been previously used to distinguish non-human ape from human *P. vivax* strains. These results suggest that both human and ape strains of *P. vivax* exist within the DRC and the apes may represent a potential reservoir of *P. vivax* for at least some human infections.

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CEREBROSPINAL FLUID CYTOKINE AND CHEMOKINE LEVELS AND NEUROCOGNITIVE FUNCTION IN UGANDAN CHILDREN WITH CEREBRAL MALARIA

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Inflammation appears to play an important role in cerebral malaria (CM), but little is known about which inflammatory factors contribute to development of CM or neurocognitive sequelae after CM. Assessment of cerebrospinal fluid (CSF) cytokines and chemokines in children with CM may provide the best available measure of the central nervous system (CNS) inflammatory process in these children. We measured CSF and plasma cytokine levels of 15 pro- and anti-inflammatory cytokines, chemokines and growth factors in 146 Ugandan children with CM and 10 North American control (NAC) children. Overall cognition, attention and associative memory were tested in children with CM 12 months after the CM episode. CSF levels of all factors were significantly higher in children with CM than in NAC. In children with CM, CSF and plasma values correlated significantly for 11 of 15 factors, suggesting that these proteins cross an impaired blood-brain barrier (BBB) into the CNS. CSF interleukin-8 (CXCL-8/IL-8) and monocyte chemoattractant protein 1 (CCL-2/MCP-1) levels were higher in CSF than plasma. Increased CSF IL-8 levels were the only factor associated with mortality (P=0.05). In children <5 years, increased CSF granulocyte-colony stimulating factor (G-CSF), IL-1 receptor agonist (IL-1ra) and MCP-1 levels correlated with worse cognitive ability, while in children ≥5 years, increased IL-1ra, MCP-1, macrophage inflammatory protein (MIP-1) and CCL5/RANTES correlated with worse cognitive ability (P value range, 0.006-0.04). No association was seen for any CSF protein with attention or associative memory. Children with CM have increased levels of pro- and anti-inflammatory cytokines and chemokines, likely due to passage from plasma to CNS across an impaired BBB, except in the case of IL-8 and MCP-1, which appear to be produced in the CNS. The association of increased CSF IL-1ra and MCP-1 with worsened cognitive scores in all ages suggests that these factors may be involved long-term brain injury in children with CM, but further study in other cohorts is needed, as these factors were not strongly associated with cognitive impairment.

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A NOVEL FACS TECHNIQUE TO MEASURE AUTOPHAGY IN *PLASMODIUM FALCIPARUM*

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Autophagy is a normal homeostatic process by which cells degrade waste. The purpose of this research project is to develop a novel FACS technique to measure autophagy in *Plasmodium falciparum*. At least 26 homologs of established autophagic proteins exist in *P. falciparum* and recent publications suggest that autophagy occurs in the parasite in response to stressors; however, a robust assay has not yet been developed for the measurement of autophagy in this parasite. A recently developed assay to measure autophagy in mammalian cells involves the use of FACS to measure autophagosomes and lysosomes using pH-specific dyes. LysoID specifically stains lysosomes by staining low pH. Although lysosomes have not been specifically identified in *P. falciparum*, LysoID appears to stain the food vacuole—the acidic compartment of *P. falciparum*—as confirmed by confocal imaging. In other organisms, CytoID stains autophagosomes by staining intermediate pH and using Atg8/LC3 as an anchor. Previous studies have observed upregulation of autophagy in *P. falciparum* following a 6-hour amino acid starvation period. We incubated 3D7 for 6 hours in amino acid-free media, and found that intraerythrocytic ring-stage parasites stained with LysoID had significantly higher mean fluorescence intensity (MFI) than those that were incubated in complete media (979.3 vs. 555.5, p<0.001). In other systems, an increased LysoID signal alone can be used as a marker of autophagy induction. CytoID stained starved intraerythrocytic parasites with slightly greater intensity than normal parasites, though this was not significant (MFI 63.0 vs. 58.1, p=0.27). This project demonstrates the potential for using FACS to measure autophagy in *P. falciparum*.

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DEVELOPMENTAL CYCLE AND TISSUE SEQUESTRATION OF *PLASMODIUM VIVAX* TRANSMISSION STAGES IN THE NON-HUMAN PRIMATE MODEL

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The recently launched global effort to eradicate malaria has been stimulated by the dramatic decrease of the disease incidence in sub-Saharan Africa. While *Plasmodium falciparum* is the deadliest of human malaria parasites, *P. vivax* is a major cause of malaria morbidity within and outside of Africa. *P. vivax* is now a major focus of the ongoing elimination agenda, with particular emphasis on development of *in vitro* culture systems and understanding of key biological features, such as latency and transmission, as a basis for better diagnosis and new interventions. Though not well studied it is believed that transmission stages, or gametocytes, of *P. vivax* take 48 hours to develop and are present in circulation throughout their cycle. They appear in blood circulation 3-5 days after the first asexual parasites are detected microscopically, and therefore transmission can occur well before the patient is symptomatic. The goal of the present study was to develop diagnostic markers to characterize the *P. vivax* transmission cycle in the *Aotus* non-human primate model and for future field studies. Comparative transcriptional analysis of *P. falciparum* versus *P. vivax* gametocytes demonstrated a conserved cascade of stage specific gene expression until maturity despite significantly different cycle length. A subset of conserved gametocyte stage-specific markers was successfully validated by quantitative Real-Time PCR (qRT-PCR) and antibody assays in peripheral blood samples from infected *Aotus* monkeys. Systematic investigation of different tissues from infected monkeys indicated an enrichment of gametocytes in the bone marrow and sub cutaneous fat by a multiplex qRT-PCR assay with our stage-specific markers. To investigate possible tissue specific sequestration of *P. vivax* gametocytes during infection, detailed histological analyses are ongoing to determine localization of specific parasite stages across the organs.

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BIOENERGETIC CHARACTERIZATION OF MUTANT *PLASMODIUM FALCIPARUM* STRAINS RESISTANT TO MITOCHONDRIAL INHIBITORS

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The *Plasmodium* mitochondrion, in particular the ETC enzymes, has been considered as a promising drug target and there have been many reports of antimalarial agents targeting cytochrome *bc1* complex and DHODH. However, there are little bioenergetic studies regarding resistant

strains against mitochondrial inhibitors and fitness cost of the mutations. Previously, we developed a robust bioenergetic assay protocol utilizing an Extracellular Flux Analyzer that enables simultaneous investigation of mitochondrial respiration and glycolysis of *Plasmodium falciparum* in a physiologically relevant microenvironment with readout of an oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Using this assay protocol, we observed OCR increase by substrates of the ETC complexes, such as succinate, glycerol-3-phosphate and dihydroorotate in saponin-freed schizont stage parasites. In this study, we first compared OCR responses to the ETC substrates between Dd2 and mutant strains, BTZ^R, ATV^R and IDI-5994^R which are resistant to cytochrome *bc1* inhibitors, benzothiazepine, atovaquone, and IDI-5994 respectively. As a result, we found that all resistant strains had smaller OCR elevation compared to their parental Dd2 and that the degree of OCR response decreased in the following order: IDI-5994^R, BTZ^R and ATV^R. IDI-5994^R strain has mutation in Qi site of cytochrome *b*, while $BTZ1^R$ and ATV^R strains have mutations in Qo site, and therefore our observation might suggest that Qo site mutations have more impact on electron transfer to cytochrome *c*. Interestingly, ECAR readout showed that glucose increased glycolytic activity more slowly in BTZ^R and ATV^R strains than in IDI-5994^R and Dd2 strains. In addition, glucose titration revealed that ECAR elevation reached a plateau with 4mM of glucose in BTZ^R strain, while 8mM is required in Dd2. These observations might indicate that BTZ^R strain enhances its dependency to glycolysis to overcome fitness cost caused by the mutation. Further bioenergetic profiling of various strains including DHODH mutant strains will be discussed.

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DEVELOPMENT OF A NOVEL MOUSE MODEL FOR PREGNANCY MAINTENANCE DURING MATERNAL MALARIA INFECTION

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Placental malaria, a severe clinical manifestation of *Plasmodium falciparum* infection observed in pregnant women, is a major cause of pregnancy loss, neonatal mortality, and severe maternal illness. Mouse models for malaria infection during pregnancy are vital to understanding the mechanisms underlying these outcomes. Here, we describe a novel mouse model for pregnancy maintenance during maternal malaria infection utilizing outbred Swiss Webster mice. When infected with *P. berghei* or *P. chabaudi* in early gestation, most mouse strains will abort their pregnancies at mid-gestation. However, outbred Swiss Webster mice infected with *P. chabaudi* AS in early gestation carry their pregnancies to term, providing a model for pregnancy maintenance during maternal malaria infection. Furthermore, as previously observed in non-pregnant mice, the gut microbiota of pregnant Swiss Webster mice influences the severity of malaria infection. Mice with 'susceptible' gut microbiota develop higher parasite burdens compared to mice with 'resistant' gut microbes. Despite the severe infections observed in 'susceptible' mice, these mice do not abort their pregnancies although litter sizes at term are reduced. Overall, this model provides a tool for exploring the mechanisms of embryo and fetal survival during maternal malaria infection, as well as the influence of the gut bacterial community on the severity of malaria infection in the context of pregnancy.

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INVESTIGATING THE ROLE OF ACS5 IN *PLASMODIUM FALCIPARUM* FATTY ACID METABOLISM

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The remarkable plasticity of the *Plasmodium falciparum* genome allows for adaptation in response to selective pressures and challenges efforts to combat this important human pathogen. Evidence of the adaptive nature of this genome includes the expansion and recent positive selection of the acyl Co-A synthetase (ACS) gene family, which includes four orthologs predicted to activate exogenous fatty acids (FAs) and play important roles in fatty acid scavenging as well as nine paralogs with unknown function. The evolutionary and functional significance for the expansion of the PfACS9 ortholog to nine paralogs, including PfACS5, is unknown. In this study we sought to functionally characterize these molecules to understand their biological role in the parasite. Using the CRISPR-Cas9 gene editing system, we successfully knocked out ACS5, a member of this expanded family. The ACS5 knockout (KO) line shows reduced growth *in vitro*. This phenotype is exacerbated by limiting growth conditions to 45% glucose supplemented with minimal fatty acids. Here, we explore this growth defect and the functional role of ACS5 in the parasite using molecular and biochemical approaches. We compare changes in the FA profile of the ACS5 KO line and its 3D7 parent. Using an LC-MS/MS approach, we profile the metabolome of the ACS5 KO and the 3D7 parent, and identify changes in key lipid species in the KO. Using these approaches, we are exploring the role of ACS5 in downstream metabolic pathways, including desaturation and elongation pathways, as well as phospholipid biosynthesis. We hypothesize that the expansion and recent positive selection of the PfACS gene family are the consequence of metabolic pressures driving parasite evolution. Therefore, understanding FA metabolism will give us insight into key metabolic pathways that might serve as potential targets for novel antimalarials.

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METABOLIC CONVERSION OF CARBOXY-PRIMAQUINE, A MAJOR METABOLITE OF PRIMAQUINE, TO POTENTIAL HEMOTOXIC INTERMEDIATES

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Plasmodium vivax malaria has broader geographical distribution than falciparum, forms dormant hard to kill hypnozoites liver stages, which can activate weeks to years after primary infection causing relapsing malaria episodes. Primaquine (PQ) is the only drug approved for prevention of malaria relapse. PQ also has activity against stage V mature gametocytes of *Plasmodium falciparum* and a key transmission-blocking agent. However, PQ causes severe hemolytic anemia in individuals with genetic deficiency of enzyme glucose 6-phosphate dehydrogenase (G6PD). Metabolism of PQ through oxidative deamination pathway generates high plasma levels of carboxyPQ (CPQ). This pathway determines characteristic pharmacokinetic properties of PQ and is primarily responsible for its short half-life. This is considered as a major therapeutic limitation of PQ, which requires a 14 days long treatment for malaria radical cure. The plasma levels of CPQ remain high even after 24 hours of treatment with PQ. CPQ has been considered to be a non-toxic and inactive metabolite of PQ. However, recent studies suggested further metabolism of CPQ through CYP mediated pathways generating potential hemotoxic metabolites. CPQ with pooled human liver microsomes (HLM) generated marked hemolytic toxicity response *in vitro*. Further, CYP profiling analysis showed that CYP1A2 and CYP2B6 were the major CYPs, which can elicit the *in vitro* hemotoxic response to CPQ, with CYP3A4 and CYP2D6 having less prominent effects. Incubation of CPQ with pooled HLM resulted in more than 35% depletion of CPQ within 2 hours. Hydroxy CPQ (m/z 291) and quinone-imine (m/z 289) were identified as the major metabolites. Interestingly, metabolism of CPQ with pooled HLM was not enantioselective. The studies confirm further metabolism of CPQ through CYP mediated pathways, which generated reactive quinone-imine CPQ and hydroxyl CPQ, the potential hemolytic metabolites. The studies indicate interesting differences in CYP mediated pathways for metabolism of PQ and carboxyPQ. These results warrant further evaluation of CPQ for the potential to cause red cell damage in G6PD-deficient individuals.

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PHOSPHORYLATION OF *PLASMODIUM* EUKARYOTIC INITIATION FACTOR 2α IN RESPONSE TO ARTEMISININ THERAPY

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Artemisinin and its derivatives are the most potent anti-malaria drugs. Nevertheless, artemisinin monotherapy is associated with accumulation of dormant ring stages and with recrudescence of *Plasmodium* infection, which is considered a treatment failure. The molecular mechanism(s) leading to parasite dormancy are under investigation. Here we report that dormancy is associated with phosphorylation of the parasite's eukaryotic initiation factor-2α (eIF2α). In an attempt to reveal which one of three *Plasmodium* kinases, eIK1, eIK2, or PK4 is involved we generated knockouts of the enzymes. Following artesunate treatment, the eIK1(-) and eIK2(-) parasites phosphorylated their eIF2α and entered dormancy like wild type. We could not obtain knockouts of PK4 because it is essential for blood stage development where the gene targeting takes place. Nevertheless minutes after drug treatment PK4 dimerized, autophosphorylated and phosphorylate eIF2α indicating that it is the enzyme that controls dormancy. Artesunate-induced dormancy is extended by salubrinal, a selective inhibitor of the eIF2α-P phosphatases, which provides evidence for the mechanism that *Plasmodium* phophorylates eIF2α causing dormancy in response to artemisinin treatment.

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EFFECTIVE SCALING-UP OF SEASONAL MALARIA CHEMOPREVENTION IN BURKINA FASO

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Burkina Faso scaled-up SMC implementation in 2015, covering 17 districts with a population of about 900,000 children under 5 years of age. Delivery, primarily door-to-door, was for four months starting late July. Each month the first dose of the 3-dose regimen was administered by a health worker and the remaining doses left with the caregiver. To evaluate the effectiveness of SMC delivery at scale, a survey was conducted at the end of the transmission season in 11 districts where SMC was delivered through the ACCESS-SMC project. 50 villages were selected with probability proportional to size, and households selected using compact segment sampling. SMC record cards were inspected and caregivers were asked about adherence, side effects, reasons for missed treatments, the time and any costs involved to obtain SMC for their child, the caregiver's level of education and socioeconomic status. Utilisation of insecticide-treated bednets by household members was also recorded. Children up to 7 years old were included in order to determine if children above the recommended age limit were being treated. Data were collected using Android tablet devices. 1000 children were surveyed. 741 of these were eligible to have received 4 cycles of SMC (aged between 3months and 5years at the first cycle). Of these 94% had received an SMC card and at least one SMC treatment. 83% received at least 3 cycles. 97% of caregivers reported that they had administered both unsupervised doses of amodiaquine the month before the survey. The mean percentage of children who attended for SMC who did not receive the treatment from the health worker, obtained from health worker tally sheets, was about 1% to 2%, mostly because the child was unwell. Of 95 children aged 6 to 7 years at the survey, 80% reported having received SMC. 89% of children slept under a bednet the night before the survey. In conclusion, a high level of coverage was achieved during 2015, in the first phase of implementing SMC on a large scale in Burkina Faso. Sustaining this achievement will be challenging.

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PREVALENCE OF MUTATIONS ASSOCIATED WITH SULPHADOXINE-PYRIMETHAMINE (SP) RESISTANCE IN *PLASMODIUM FALCIPARUM* SAMPLES FROM THE GENERAL POPULATION AND PREGNANT WOMEN IN NANORO, BURKINA FASO

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Pregnant women are at increased risk of *Plasmodium falciparum* infection, which can result in maternal anemia, low birth weight babies and other sequelae. Most Sub-Saharan African countries have therefore implemented intermittent preventive treatment for pregnant women (IPTp) using sulphadoxine-pyrimethamine (SP). However, concerns are rising about its continuous efficacy because of increasing resistance against SP. Point mutations in the *Dhps* and *Dhfr* genes of *P. falciparum* are associated with resistance, especially combinations like the triple *Dhfr* mutant (51I, 59R, 108N), the double *Dhps* mutant (437G, 540E) and moreover the quintuple mutant. The aim of our study was to estimate the current levels of SP resistance in Nanoro, Burkina Faso and to test whether the mutation rate increases during pregnancy. Filter paper samples from pregnant women at first antenatal care visit (ANC1) and at delivery were collected from March 2014 till September 2015 as part of an intervention trial (COSMIC). Furthermore, samples from the general population (GP) were collected from March till May 2015. DNA was extracted and *P. falciparum* positive samples were detected by qPCR. Next, nested PCR was used to amplify the *Dhps* and *Dhfr* genes and products were sent for sequencing. We found a high prevalence of *Dhfr*-51, -59 and -108 overall, with a trend of higher levels in GP and delivery samples compared with ANC1 samples (70.7%, 74% and 61.5% triple *Dhfr* mutants respectively). Statistical analyses will follow, but this trend could possibly indicate selection of resistant parasites during pregnancy. However, the concurrent higher mutation rate in GP samples needs to be explored. *Dhps*-437 also showed high mutation rates (89.4%, 84% and 83.4% respectively) without a clear trend. The *Dhps*-540 mutation was found in one GP sample and in two delivery samples, of which one was a quintuple mutant. To our knowledge this is the first time the *Dhps*-540 mutation is found in Burkina Faso. This finding and the high prevalence of the other mutations raises concerns about efficacy of IPTp-SP in the future. Other drug combinations to tackle malaria in pregnancy should therefore be explored.

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ON THE ADEQUACY OF A 28 DAY FOLLOW-UP PERIOD FOR ARTEMETHER LUMEFANTRINE AGAINST UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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Clinical trials are the gold-standard for deriving information on antimalarial efficacy from which policy decisions can be made. These estimates are vulnerable to methodological approaches such as the duration of study and loss to follow-up. The aim of this work is to explore the optimal duration of follow-up for capturing PCR confirmed recrudescences following treatment with artemether-lumefantrine (AL), and to assess the sensitivity of the recommended minimum follow-up duration of 28 days in patients from Africa and Asia. The cumulative baseline hazard, estimated from Cox regression models with shared frailty on study-sites and fractional polynomials to capture nonlinear associations, was used to estimate the probability density of recrudescences. The area under the

density curve (AUC) was calculated to determine the optimal follow-up period; accuracy of which was evaluated using simulation techniques. Data were available from 54 efficacy trials on AL (n=7,735; 2002-2014) in children less than 5 years in Africa and 10 trials in Asia in patients of all ages (n=1859, 2000-2010) with a minimum follow-up of 28 days. There were 221 (2.9%) recrudescent infections in Africa and 41 (2.2%) in Asia within 63 days. In studies with follow-up duration of 42 days or longer, 43% (47/109) of these recrudescences in Africa and 24% (10/41) in Asia were missed with a day 28 follow-up. The missed proportions were even higher when estimated using the AUC approach, which makes use of all available data. A shift to the left of the probability density function (i.e. recrudescences occur earlier) was observed for Asia compared to Africa. Effects of baseline parasitaemia and treatment dose on the shape and location of the density function were also studied. This pooled analysis confirms that the current recommended follow-up duration of 28 days remains inadequate for accurately determining AL efficacy and fails to identify an estimated 62% of the recrudescences in Africa and 49% in Asia. The feasibility and cost-effectiveness of a longer follow-up duration warrants further investigation while also considering misclassification errors associated with genotyping.

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PLASMODIUM FALCIPARUM PARASITE CLEARANCE IN THE PERUVIAN AMAZON AS PART OF A DOD HARMONIZED CLINICAL TRIAL

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There is a global threat of *Plasmodium falciparum* resistance to artemisinin-based combination therapies observed in South-East Asia. Three DoD laboratories in Kenya, Peru and Thailand conducted a harmonized clinical trial to determine parasite clearance time after receiving artesunate. Data from Peru is presented.Participants between 5-65 years old with uncomplicated, microscopy confirmed, *P. falciparum* mono-infection, with asexual parasite density between 1,000 - 100,000 parasites/µL were enrolled. Participants were hospitalized for three days and received 4 mg/kg artesunate on Days 0, 1 and 2; 15 mg/kg mefloquine on Day 3, and 10 mg/kg mefloquine on Day 4. The parasite clearance rates were determined by microscopy every 4 h during first 12 h and then every 6 h until 72 h after first being treated with artesunate. Clinical and parasitological responses were assessed for 42 days.Between June 2014 and November 2015, 482 people with *P. falciparum* mono-infection were identified at eight health centers in Iquitos, in the Peruvian Amazon Basin, but most did not comply with the study requirements. Seventy-four subjects were consented and screened, and among them, 55 subjects with uncomplicated *P. falciparum* malaria were enrolled. Two participants were excluded due to serious adverse events and mixed infection. Participants cleared parasitemia by hour 42 (PCR uncorrected).The mean age of participants was 31.2 (95% CI 26.8 - 35.6), and 22 (41.5%) were female. The geometric mean parasite count at admission was 5514 parasites/µL (95% CI 4095-7426). The clearance rate constant (by hour) median was 0.32 (IQR: 0.29-0.39). The slope half-life median is 2.18 hours (IQR: 1.78-2.39). Finally, 50% and 99% parasite clearance median times (PC50 and PC99) were 5.84 (IQR: 3.07-7.28) and 17.02 (IQR: 15.22-19.35) respectively. All participants completed Day 42 follow-up and met the adequate clinical and parasitological response endpoint.No suggestion of resistance to artesunate was found among the participants evaluated in the Peruvian Amazon. However, surveillance using molecular markers such as K13 should be used as a complementary regional strategy.

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DOES METHYLENE BLUE ENHANCE THE *EX VIVO* ANTIMALARIAL BLOOD SCHIZONTOCIDAL ACTIVITY OF ARTESUNATE-AMODIAQUINE?

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Reports of falciparum malaria patients in Cambodia and Vietnam failing treatment with dihydroartemisinin-piperaquine highlights the urgent need to contain and reduce the spread of artesunate based combination therapy (ACT) resistant strains. As part of this effort a triple drug strategy approach should be investigated to extend the useful life of ACTs. The objective of the present study was to determine whether methylene blue (MB) can enhance the pharmacodynamic (PD) *ex vivo* antimalarial activity of artesunate-amodiaquine (ASAQ). If ASAQ+MB can be demonstrated to be more potent than ASAQ alone, then the triple combination may provide a better option to treat ACT resistant malaria infections. In an open labelled, randomized cross-over design, a single oral dose of either ASAQ (2 tablets, with each tablet containing 100 mg AS and 270 mg AQ) or ASAQ (2 tablets)+MB (5 tablets, with each tablet containing 65 mg MB) was administered to 16 healthy Vietnamese volunteers. After an 8 week washout period the same participants received the alternative drug combination. Serial blood samples were collected up to 28 days after the last dose of either ASAQ or ASAQ+MB. The *ex vivo* antimalarial activity of ASAQ and ASAQ+MB was assessed by subjecting the participant's plasma samples collected after drug administration against an artemisinin-sensitive and an artemisinin-resistant *Plasmodium falciparum* line *in vitro*. Based on the participant's plasma inhibitory concentration profiles the preliminary *ex vivo* data revealed MB to enhance the blood schizontocidal activity of ASAQ by at least 2-fold against both *P. falciparum* lines. Additionally, using LC/MS/MS we determined the pharmacokinetic (PK) properties of the partner drugs including their principal active metabolites. The PK-PD relationship of ASAQ and ASAQ+MB will be compared and their implications for the treatment of multidrug-resistant falciparum malaria will be discussed.

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PARASITE CLEARANCE AND DECLINES IN ARTEMETHER EXPOSURE OVER THE COURSE OF ARTEMETHER-LUMEFANTRINE TREATMENT FOR *PLASMODIUM FALCIPARUM* MALARIA IN UGANDAN CHILDREN

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We sought to assess the association between parasite clearance parameters and artemether (AR) and dihydroartemisinin (DHA) pharmacokinetic (PK) exposure in HIV-infected and HIV-uninfected children in Uganda treated with artemether-lumefantrine (AL) for malaria. Children ≤8 years underwent intensive PK sampling post-1st dose of AL followed by every 12-hour blood smears. AR and DHA exposure was compared to post-last AL dose PK in concurrently enrolled children using non-compartmental analysis. Parasite clearance parameters were calculated using the WWARN Parasite Clearance Estimator. Post-1st and last dose PK parameters were estimated in 103 children (77 HIV-uninfected and 26 HIV-infected) and 142 children (51 HIV-uninfected and 91 HIV-infected), respectively. In HIV-uninfected children, post-last dose AR area-under-

the-curve from 0 to 8 hours (AUC) was 3-fold lower as compared to the AUC post-1st dose of AL (ratiolast/first dose 0.31; p<0.0001), while DHA exposure increased (AUC ratiolast/first dose 1.75; p=0.0003). Additionally, AR exposure post-1st dose was 3 to 6-fold lower in HIV-infected children on efavirenz and nevirapine compared to HIV-uninfected children (AUC ratio 0.16; p<0.0001 and AUC ratio 0.35; p=0.001, respectively). Post 1st-dose DHA exposure was similarly lower in efavirenz-treated vs HIV-uninfected children (AUC ratio 0.45; p=0.028). Parasite clearance slope half-life was significantly longer in HIV-infected (3.51 hrs; 95% CI 2.98, 4.03) vs HIV-uninfected children (2.8 hrs; 95% CI 2.38, 3.36); p=0.003. AR exposure demonstrates a significant time-dependant decrease following the 1st dose of AL in all malaria-infected children, with those on efavirenz exhibiting dramatic reductions to AR and DHA throughout the dosing interval. Parasite clearance parameters are also prolonged in HIV-infected vs HIV-uninfected children. These findings have important implications for AL efficacy and the risk of selection for artemisinin resistance in this vulnerable population. Multivariate regression to explore the relationship between PK and parasite clearance parameters is underway and will be presented.

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DEFINING THE DESIRED ATTRIBUTES OF NEXT GENERATION SEASONAL MALARIA CHEMOPREVENTION (SMC) DRUG

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Seasonal Malaria Chemoprevention (SMC) is implemented using sulfadoxine-pyrimethamine (SP) and amodiaquine (AQ) in areas where malaria transmission and 60% of clinical malaria cases occur during a short transmission period (~4 months), and where SPAQ remains efficacious (>90% therapeutic efficacy) -in the Sahel sub-region of Africa.Medicines for Malaria Venture (MMV) is working to identify alternative molecules with chemoprevention properties that may be suitable as next generation SMC tools. To help refine the target product profile (TPP) for potential new medicines, MMV commissioned a survey to identify the preferred attributes of next generation SMC drugs.Research focused on The Gambia and Burkina Faso, countries that have successfully implemented two SMC campaigns using a door to door approach, with >90% coverage. A varied range of international, regional and local interviewees (n=112) were selected to provide input on three TPPs in the context of a hypothetical scenario in which SPAQ would no longer be effective for chemoprevention.The TPPs tested contained fourteen attributes including indication, efficacy, dosing, administration and taste; each TPP varied in its protective efficacy and administration regimen. For each TPP, participants were asked to score the overall product and each product attribute on a scale from 1 to 5.Out of this survey, four attributes were emphasized for the development of new SMC products: (1) A well tolerated product, suitable for MDA; (2) protective efficacy at least equal to current SPAQ; (3) a child-friendly formulation to facilitate administration and adherence; (4) a monthly administration schedule to support the current effective door-to-door campaign. A fixed distribution point closer to the community, with a weekly administration could be considered if incentivized by a high protective efficacy (>80%). An alternative with a single injectable administration (before rainy season) and protective efficacy levels greater than 75% were included in the survey. Interviewees generally considered an injection to be most effective and to offer less logistical stress.

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TARGETING ADENYLATE CYCLASE AS A NOVEL AVENUE FOR ANTIPARASITIC DRUG DESIGN

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Cyclic AMP (cAMP) is an essential, and highly conserved, secondary messenger molecule with critical signaling roles in all eukaryotes. While the function of cAMP is largely conserved, the proteins within the cAMP signaling pathway possess significant structural differences across organisms. Here, we used directed evolution and comparative chemogenomics in both *S. cerevisae* and *Plasmodium falciparum* to show that a novel class of antimalarial compounds, the phenyl-amino-methyl-quinolinols (PAMQs), specifically target the homodimeric class of adenylate cyclases (ACs), the enzyme(s) responsible for cAMP synthesis in fungi and protozoa parasites. We isolated *S. cerevisiae* resistant against two MMV malaria box compounds, MMV0570 and MMV7181, which harbored mutations in several members of the cAMP pathway, including *cyr1*, the *S. cerevisae* homolog of AC. In *P. falciparum*, we found that these compounds both possess potent (20-50 nM) activity against asexual and sexual blood stages of *P. falciparum* and strongly inhibited parasite cAMP levels. This analysis extended to 113 highly related analogs, which identified several additional compounds with strong antimalarial activity and inhibition of parasitic cAMP levels. The antiparasitic mechanism of action of these compounds was further interrogated via *in silico* molecular docking of the PAMQs with AC, recombinant expression of *P. falciparum* AC and genetic engineering to manipulate the expression level of the two parasite ACs. These compounds also show activity against several additional human pathogens that contain homodimeric ACs, including numerous fungi, *Trypanosoma cruzi* and *Leshmania infantum*. Importantly, we show that the PAMQs are highly selective, as they do not significantly affect cAMP levels in human cells. This specificity for homodimeric AC suggests that the cAMP/AC pathway is a promising pathway to target for chemotherapeutic intervention against these parasitic species. Given the increasing lack of effective antiparasitic drugs, the identification of the cAMP pathway as a candidate for small molecule intervention represents a promising new avenue for drug development.

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KELCH PROTEIN GENE (K13) MUTATIONS IN *PLASMODIUM FALCIPARUM* POPULATIONS IN THREE MALARIA HOT SPOTS OF VIETNAM

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Malaria remains a public health challenge in Vietnam despite a substantial reduction in the incidence of disease over the last twenty years. Moreover, the spread of artemisinin resistance of *Plasmodium falciparum* which compromises the therapeutic efficacy of artemisinin combination therapies is the great threat to current global initiatives to control and eliminate malaria. An understanding of genetic factors that determine how it emerges and spreads is necessary. K13 propeller polymorphism mutations are important determinants of artemisinin resistance.In the study, propeller domain gene of K13 were successfully sequenced in 1060 isolates collected in 3 malaria hot spots of Vietnam from 2009-2016. Ten genotypes of K13 were found including 8 mutations (T474I, Y493H, R539T, I543T, P553L, C580Y, V568G and P574L) after the position of

440th amino acid. The prevalence of K13 mutations were 29%, 6% and 44% in each hot spot Binh Phuoc, Ninh Thuan, Gia Lai respectively. The most important C580Y became dominant genotype in recent year with 81% in Binh Phuoc and 67% in Gia Lai Province. There is the association between K13 mutations and prolonged parasite clearance half-life. Identification of K13 mutations and its frequency in population of *P. falciparum* in Vietnam will support surveillance efforts to contain, prevent the artemisinin resistance and facilitate the development of effective strategy to combate drug resistance.

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INTERMITTENT PREVENTIVE TREATMENT CONTINUES TO PROVIDE BENEFIT TO MALAWIAN PREGNANT WOMEN

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Drug resistance, particularly the *Pfdhps*-581G mutation, when present with the quintuple *dhps/dhfr* mutant haplotype, may undermine the efficacy of intermittent preventive treatment in pregnancy with sulphadoxine-pyrimethamine (IPTp-SP). We conducted a cross-sectional study at delivery in an area with high prevalence of SP resistance in order to assess whether IPTp-SP remains efficacious. HIV-uninfected women with singleton pregnancies were enrolled at delivery from June to October, 2015 at two sites in southern Malawi. Demographics, clinical data, peripheral blood, and placental samples were collected, and infants were examined. Samples were tested for malaria using rapid diagnostic tests (RDT), microscopy, and polymerase chain reaction (PCR); PCR positive samples will be genotyped for mutations at *dhps*-540 and *dhps*-581. We enrolled 506 women: mean age was 24.2 years (range 15-45); mean gestational age at delivery was 39.4 weeks (range 28-43); 35% were primigravid, 17% were secundigravid, and 48% were multigravid; 81% owned an insecticide treated net, 65% of whom slept under it on the previous night. 11% received no IPTp, while 6%, 24%, and 58% received 1, 2, and 3 or more doses, respectively. Overall, 18% had evidence of malaria: 10.5% by RDT, 7.7% by peripheral smear, 5.6% by placental smear, and 15% by PCR. Malaria was more common among women who received <3 vs ≥3 doses of IPTp-SP: 15% vs 7%, p=0.003 by RDT; 10% vs 5%, p=0.04 by maternal peripheral smear; 7% vs 5%, p=0.33 by placental smear; and 14% vs 16%, p=0.69 by PCR; 20% vs 17%, p=0.42 for any malaria. Birthweight was significantly higher among women who had received ≥3doses of IPTp-SP (3121gm) compared to those who received <3 doses (3032gm, p=0.03). IPTp-SP continues to provide benefit to Malawian pregnant women, with a significantly higher mean birthweight and less maternal malaria among women who received 3 doses; the effect on patent parasitemia was greater than on malaria detected by PCR. Additional data on the prevalence of *Pfdhps*-581G and the relationship of this mutant to birth outcomes will also be presented.

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UNCOMPLICATED MALARIA TREATMENT FAILURES AFTER ARTESUNATE-AMODIAQUINE COMBINATION THERAPY IN TWO ECOLOGICAL ZONES IN GHANA

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Continuous monitoring of the therapeutic efficacy of artemisinin-based combination therapy for the treatment of uncomplicated malaria has become critical, within the context of malaria control, in an era of the development and spread of artemisinin resistance. The therapeutic efficacy of artesunate-amodiaquine (AS-AQ) was studied from June to September 2015 among children, aged 6months to 14 years, reporting with uncomplicated malaria at two sentinel health facilities in the forest and coastal zones of Ghana. A total of 237 children were recruited for the study: 110 in the forest zone and 127 in the coastal zone. These children were followed up for 28-days using the 2009 WHO protocol for monitoring antimalarial drug efficacy. Preliminary results show no early treatment failure in the 2 ecological zones. Overall pcr-uncorrected late clinical and parasitological failure rates were 6.7% (95% CI: 3.0-13.7) in the forest zone and 11% (95% CI: 6.4-18.1) in the coastal zone (p=0.357). There were no significant differences in treatment failure rates between children aged less than 5 years and children aged 5-14 years in both ecological zones. The main adverse event reported in the 2 ecological zones was vomiting. Prevalence of vomiting in the forest zone was 6.4% (95% CI: 2.8-13.2) on day-0; 6.5% (95% CI: 2.9-13.4) on day-1, and 10.3% (95% CI: 5.5-18.0) on day-2 (p=0.474). Prevalence of vomiting in the coastal zone was 5.5% (95% CI: 2.4-11.4) on day-0; 0.8% (95% CI: 0.1-5.0) on day-1; and 2.4% (95% CI: 0.6-7.3) on day-2 (p=0.076). We conclude that AS-AQ remains efficacious and safe for the treatment of uncomplicated malaria in the forest and coastal zones of Ghana.

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DOSE-RESPONSE EFFECT OF SULFADOXINE-PYRIMETHAMINE ADMINISTERED AS INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN PREGNANCY REDUCES ADVERSE BIRTH OUTCOMES RELATED TO SEXUALLY TRANSMITTED AND REPRODUCTIVE TRACT INFECTIONS

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The World Health Organization recommends intermittent preventive treatment with sulphadoxine-pyrimethamine for pregnant women resident in areas of moderate (stable) or high malaria transmission at every scheduled antenatal care visit from the second trimester until delivery to prevent the adverse consequences of malaria infection during pregnancy. A prospective cohort study was conducted between November 2013 and April 2014 among 1086 pregnant women attending antenatal care facilities in the Nchelenge District of Zambia. Recipients of ≥ 2 doses of intermittent preventive treatment were one-half as likely to have any adverse birth outcome - a composite measure of (1) stillbirth, (2) low birthweight, (3) preterm delivery, or (4) intrauterine growth retardation - compared to recipients of 0-1 dose (adjusted odds ratio [OR] 0.49; 95% confidence intervals [CI] 0.33, 0.75). In the sub-population of women who had an adverse birth outcome, odds ratios for mono- and co-infection with malaria and/or curable sexually transmitted and reproductive tract infections (STI/RTI) were also lower among women who received ≥ 2 doses versus 0-1 dose: malaria mono-infection (OR 0.25; 95% CI 0.09, 0.68); malaria plus trichomoniasis or bacterial vaginosis (OR 0.89; 95% CI 0.44, 1.83); trichomoniasis or bacterial vaginosis (OR 0.85; 0.38,

1.91); gonorrhoea or chlamydia (OR 0.08; 95% 0.01, 0.80); syphilis and malaria or other curable STI/RTI (OR 0.34; 0.14, 0.85). This dose-response protective effect was observed consistently across the four individual adverse birth outcomes, and in 25 of 28 categories of malaria and curable STI/RTI mono- and co-infection. Interestingly, ≥ 2 doses compared to 0-1 dose reduced the odds of an adverse birth outcome even among pregnant women who had neither malaria nor curable STIs/RTIs (OR 0.34; 0.14, 0.85), suggesting that sulfadoxine, a broad-spectrum antimicrobial drug, is protective against pathogens beyond malaria and curable STIs/RTIs.

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POPULATION GENETICS OF THE CHLOROQUINE-RESISTANT GENE PFCRT IN CAMEROONIAN FIELD *PLASMODIUM FALCIPARUM* ISOLATES

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Understanding the population genetics of genes which shape resistance to antimalarial drugs can help in devising novel control strategies. One of the major hurdles in malaria control lies on the evolution and dispersal of the drug-resistant malaria parasite, *Plasmodium falciparum*. Specific mutations in the *P. falciparum* chloroquine resistant transporter gene "Pfcrt" have been associated with resistance to not only chloroquine, but also to amodiaquine, one of the artemisinin partners used in Cameroon for the treatment of uncomplicated malaria. We here present data on genetic variation at the single nucleotide polymorphisms (SNPs) level in the Pfcrt gene in five distinct geographical settings of the Southern-Cameroon (the most malaria endemic part), i.e. Ebolowa, Yaounde, Bertoua, Douala and Kye-ossi (a city bordering Cameroon and two others African countries). Two novel mutations, hitherto unreported (in Cameroon) were found in the Pfcrt gene and variable genetic diversity was observed across the populations. High linkage disequilibrium was found between few SNPs in all the populations traducing a synergic work for conferring/maintaining a higher level of resistance. The inference of evolutionary pattern of this gene in Cameroon based on genetic diversity data depicts a signature of Darwinian positive natural selection on these loci. While observation of novel mutations might traduce new varieties in chloroquine/or amodiaquine resistance (proposal awaiting an experimental verification), signal of positive selection can be the result of drug pressure exerted by misuse of chloroquine (though officially banned from the country) and/or amodiaquine. Our findings thus, provide a baseline understanding of the evolution of a malaria drug resistant gene in Cameroon and suggest a successful establishment of chloroquine-resistant strains which requires urgent attention of malaria control programme in Cameroon.

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THE PROTECT STUDY: MATERNAL AND CHILD MALARIA CHEMOPREVENTION TO ENHANCE CHILD DEVELOPMENT

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More than 30 million pregnancies occur annually in *Plasmodium falciparum* malaria endemic areas. The systemic and placental changes that occur with malaria in pregnancy could adversely affect the developing fetal brain, which could in turn affect long-term childhood neurodevelopment (ND). Asymptomatic malaria, uncomplicated malaria and severe malaria in childhood have also been associated with ND deficits. However, the effects of maternal and child malaria chemoprevention on child ND have not been assessed to date. We assessed child ND at ~1 year of age in Ugandan children enrolled in a randomized, double-blinded, clinical trial of maternal and child malaria chemoprevention. In this trial, 300 pregnant HIV-uninfected women were randomized at 12-20 weeks of gestation to malaria chemoprevention with 3 doses of sulfadoxine-pyrimethamine, 3 doses of dihydroartemisinin-piperaquine (DP), or monthly DP, and their children were then randomized to receive DP chemoprevention monthly or every 3 months from 2 to 24 months age. Of the 272 children still in the study and eligible for testing, 193 have been assessed for ND outcome at ~1 year of age (mean (SD) 12.6 (0.6) months) using the Bayley Scales of Infant and Toddler Development (Third Edition). To date, the mean (standard deviation) composite scores for Cognition, Language and Motor scales are 103.2 (12.55), 99.47 (9.38) and 105.4 (12.51) respectively. There is no difference in test performance by sex. Treatment arm allocation remains concealed until June 2016, when the one year assessments will be completed. We hypothesize that monthly DP in both mothers and children will be associated with better ND outcomes than the other arms. The final one year results of the PROTECT study, the first prospective study of child ND outcomes after maternal and child malaria chemoprevention, will be presented at the national meeting of the American Society of Tropical Medicine and Hygiene.

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THE EFFECT OF ARTEMISININ-BASED COMBINATION THERAPY (ACT) OPTIONS ON HEMATOLOGICAL RESPONSE IN *PLASMODIUM FALCIPARUM* MALARIA: A SYSTEMATIC REVIEW AND POOLED ANALYSIS OF INDIVIDUAL PATIENT DATA

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Malaria-associated anemia has a complex etiology related to increased red cell destruction and hemopoeitic suppression, compounded by malnutrition and helminth carriage. Recent reports describe variable reductions in hemoglobin after treatment of *P. falciparum* (Pf) with different ACTs, but precise quantification of the hemoglobin fall attributable to ACTs has not been evaluated widely. Understanding the normal hematological response and recovery following treatment of uncomplicated Pf malaria is crucial to quantify the risks and benefits of different ACTs and other antimalarials such as primaquine, a drug with a potentially important role in malaria elimination.A systematic search of literature databases was conducted to identify studies published from 1990 to June 2015 in which hematological data were recorded in Pf malaria patients before and after treatment with artemether-lumefantrine, dihydroartemisinin-piperaquine, artesunate (AS)-amodiaquine or AS-mefloquine. The WorldWide Antimalarial Resistance Network (WWARN), in collaboration with relevant investigators, organized an individual patient data pooled analysis standardizing and collating nearly 200 studies, with over 72,000 patients of which 70% from African countries. An a priori data analysis plan was developed to identify factors associated with anemia prevalence and hemoglobin changes following treatment with an ACT.The full analysis will be presented, including the contributions of asexual parasitemia, age, transmission intensity and drug concentration. The effect of different ACTs on hemoglobin changes (absolute and fractional) in the 7 days following treatment will be examined in relation to ACT regimen, parasite clearance time, transmission intensity and human host factors. The results of this study will be critical for better assessing safety issues and guiding the optimal therapeutic strategies for regional malaria elimination efforts.

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ANTIMALARIAL DRUG-RESISTANCE: WHAT DO HIV AND IMMUNITY HAVE TO DO WITH IT?

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The rise and spread of drug resistant malaria parasites is one of the major challenges for malaria control, and indeed will be a huge obstacle for malaria eradication. Successful drug treatment is dependent on both the killing effect of the drug and the killing effect of the immune system. In addition, the immune system is known to play an important role in within-host competition between parasites, which in turn has been shown to be a key part of resistance evolution. Moreover, there are the historical observations that resistance initially occurs in area of low transmission intensity and hence low level of antimalarial immunity. It is thus hypothesized that the immune system is a critical factor in the emergence and spread of drug resistant mutants. If immunity indeed plays a role, this has significant implications for malaria elimination where reduced immunity is a natural consequence yet this is achieved by using a high amount of drug pressure. Using data of clinical trial on IPTp use in pregnant women in Benin, Gabon, Kenya and Mozambique, we present the occurrence of resistant mutants in a variety of immune contexts: (i) HIV-co-infections, (ii) women with different levels of antibody titers, (iii) placental and peripheral infections, and (iv) primigravidae and multigravidae women.

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EFFICACY OF ARTEMETHER-LUMEFANTRINE FOR TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CRUZEIRO DO SUL, ACRE, BRAZIL

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Plasmodium falciparum malaria has high morbidity and mortality; there were 143,551 cases in the Brazilian Amazon Region in 2014, of which 15.9% were *P. falciparum* monoinfection. Artemether-lumefantrine (AL) is the first-line treatment. Following the World Health Organization recommendation to routinely evaluate antimalarial treatment policies, we are conducting a therapeutic efficacy *in vivo* trial of AL for treatment of uncomplicated *P. falciparum* malaria in Cruzeiro do Sul, Acre, Brazil from December 2015 to May 2016. The objectives of this study are to evaluate the efficacy of the first-line antimalarial regimen for treatment of uncomplicated *P. falciparum* malaria and ensure effective case management practices are maintained in Brazil. Febrile participants ≥5 years old with microscopically confirmed *P. falciparum* monoinfection with parasitemia between 250 and 200,000 asexual parasites/microliter were enrolled and treated with a supervised 3-day course of AL, dosed according to Brazilian guidelines for malaria control. Clinical and parasitological parameters are monitored for 28 days. Recrudescence is differentiated from reinfection by comparing parasite genotypes from Day 0 and the day of failure. Genetic markers associated with artemisinin resistance, including the K13 gene mutation will be assessed. A total of 127 patients have been screened, and all 85 participants have been enrolled. Four participants were excluded after enrollment; two due to parasitemia below inclusion range, one due to *P. vivax* infection upon slide review, and one due to absence of fever in the previous 48 hours. Among enrolled patients, 61 have completed follow-up, 17 are still being followed and three have been lost to follow-up. No patient has met criteria for treatment failure, although one participant was diagnosed with *P. vivax* on day 28. Patient follow-up will be completed by May, 2016. Final results will be presented. Preliminary results suggest that AL remains highly efficacious for treatment of uncomplicated *P. falciparum* in the Brazilian Amazon Region. These results will be corroborated by molecular testing for the K13 resistance gene.

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SHOULD WE STILL USE QUININE IV FOR SEVERE MALARIA?

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Malaria represents a significant health problem in patients coming back from endemic areas. Severe malaria is life threatening and the acute respiratory distress syndrome (ARDS) is among the more serious complications that invariably leads to death. We have noted the occurrence of ARDS in a few patients with severe malaria being treated with quinine IV, the only available recommended drug at the time. The question of whether quinine IV is a triggering or contributing factor of ARDS in those cases was raised. A retrospective analysis of several cases of malaria was initiated and a literature search was done. Both outcomes were not definitive in corroborating evidence against quinine IV, but the suspicion remains. In the USA, quinine IV has not been available or used in severe malaria for over 20 years! Quinidine is available in the USA. In the era of better antimalarial therapy, especially with the recommended relatively recent and effective artemisinin derivatives, or the fairly safer quinidine, should quinine IV still be listed among the treatments or used for severe malaria?

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SAFETY AND TOLERABILITY OF DIHYDROARTEMISININ-PIPERAQUINE AS INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN A REFUGEE CAMP, ADJUMANI, UGANDA

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The use of dihydroartemisinin-piperaquine (DP) is increasing in sub-Saharan Africa, though safety data in Africa is sparse. DP was used by Médecins Sans Frontières in an Intermittent Preventive Treatment (IPT) program in a refugee camp in northern Uganda in 2015. All children aged 6 months to 14 years resident in the camp were eligible for participation in the program, which consisted of three mass distributions of DP at 8 week intervals. Weight-based dosing following 2014 guidelines was used and a total of 40 611 doses of DP were administered during 3 distributions. A health-center based pharmacovigilance system was implemented during the program, and an existing community-based mortality surveillance system was continued. Signs and symptoms of both common and severe side effects due to DP were part of key sensitization messages during the campaign. Participants experiencing any symptoms were encouraged to present to the health centers in the camp, where free health care was provided. A total of 56 adverse events (AE) were reported during the 24 week follow-up period. All AEs were reported in the 10 days following DP administration. Of the 56 AEs, 28 were judged to be probably or definitely related to DP; the most common symptoms were rash or itching (12) and vomiting (6). One case of urticaria was notified. Symptom severity was noted for all AEs regardless of causality: 75% were mild and 25% were moderate. One SAE was reported: an unexplained death in the community which occurred in a 12-year old girl who was diagnosed with varicella 12 days after taking the first distribution of DP. On the 8th day of her

varicella, she developed swelling of her face and limbs over several hours, lost consciousness at home, and was pronounced dead on arrival to the health center. Her mother reported her taking only paracetamol and using a zinc oxide cream in the days prior to her death. The SAE was considered possibly related to DP, but the concomitant varicella provides an alternative explanation. These data show that DP is well-tolerated and safe when given in repeated doses in an IPT setting. Its use in similar contexts in sub-Saharan Africa should be considered.

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FALSE SECURITY FROM OBSOLETE MALARIA DRUG-RESISTANCE MARKERS

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Malaria kills 500,000 people every year and this toll may increase if *Plasmodium* parasites evolve resistance to the artemisinin combination therapies that are the current mainstay of treatment. For the last two decades, artemisinin derivatives have been the frontline treatment for malaria-infected individuals and have prevented the public health catastrophes that followed the previous failure of chloroquine. To protect them as potent antimalarials for as long as possible, Artemisinin Combination Therapy (ACT) pairs artemisinins with other classes of antimalarials with the presumption that simultaneous development of resistance to all ACT components is improbable. Despite this approach, artemisinin resistance has recently been established in Southeast Asia. It is now important to understand how parasite populations may acquire resistance to ACTs. Here we report an ominous finding: *P. falciparum* isolates collected in Southwest India in 2012 displayed antifolate resistance in cell-based assays that was as high as the most resistant parasites in the world, but these parasites did not contain the full set of classic DNA sequence markers for the highest antifolate resistance. Parasites from regions using artemisinin-antifolate combinations for decades display novel mechanisms of antifolate resistance that escape routine surveillance methods. The unwitting use of ineffective partner drugs threatens to undermine the last remaining effective antimalarial.

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ASSESSMENT OF THE USE OF MALARIA RAPID DIAGNOSTIC TESTS IN HEALTH FACILITIES IN GHANA

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Malaria rapid diagnostic tests (RDTs) have become the mainstay diagnostic tool for acute malaria infection in most health facilities in Ghana. However, in spite of the generally good performance of these tests, their use by health care providers at health facilities can be challenging. Data on the performance, reading and interpretation of RDTs under routine program conditions are limited. Since 2012, the US President's Malaria Initiative (PMI) has supported the MalariaCare partnership to collaborate with the National Malaria Control Program (NMCP) to improve quality of malaria diagnosis using RDTs in health facilities. As part of MalariaCare's quality assurance program for case management, trained clinicians and laboratory staff act as supervisors who conduct outreach training and supportive supervision (OTSS) visits to health facilities in five of Ghana's ten regions. These visits focus on skills observation and on-the- job mentoring and problem solving, with the primary aim of improving clinical assessment and evaluation skills, preparation and accuracy of malaria diagnostic tests, and adherence by clinicians to test results. A key component of the OTSS system is observing facility staff perform RDTs and providing coaching to staff on weaknesses and errors observed. Following four rounds of OTSS data tracking in focus regions, 7,502 RDT tests observed gathered information on each step in the preparation and reading of an RDT performance during 5,088 health facility visits. MalariaCare will present the findings on common errors made in conducting RDT tests, but also track progress over time in addressing these weaknesses.

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STRUCTURAL AND FUNCTIONAL EFFECTS OF HEME BINDING TO RCPFHRP2: IMPLICATIONS FOR MALARIA DIAGNOSIS

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Early diagnosis of malaria is a key element of elimination strategies because individuals with low parasite loads can serve as transmission reservoirs. Rapid diagnostic tests (RDTs) are often used in widespread malaria screening efforts to quickly and easily detect the malaria biomarker PfHRP2, a histidine-rich protein produced by the malaria parasite *Plasmodium falciparum*, in a small drop of blood. However, PfHRP2 protein sequence variation coupled with manufacturing issues can make these tests unreliable. This work is focused on investigating the structure of PfHRP2 and its relationship to the effectiveness of current RDTs. Past studies have indicated that PfHRP2 may play a role in the parasite's heme detoxification process by binding free heme and promoting its crystallization into hemozoin. We hypothesize that heme-bound protein adopts a different conformation from free protein, and that this conformational change may affect protein binding to antibodies on an RDT. This is especially a concern since native protein will be exposed to heme in blood, but purified recombinant protein used for industrial antibody production will not. This work investigates the conformational changes of rcPfHRP2 in the presence of heme using circular dichroism (CD), and the effects of heme on antibody-based PfHRP2 detection using ELISA and RDT formats.

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IMPROVING QUALITY OF MALARIA RAPID DIAGNOSTIC TESTING AND TEST ADHERENCE THROUGH QUALITY ASSURANCE

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As malaria prevalence in Tanzania declines, universal diagnostic testing for malaria becomes increasingly important to identify and manage other causes of febrile illness and reduce the threat of antimalarial resistance. In 2013, the Tanzania National Malaria Control Programme (NMCP) revised the National Guidelines for Diagnosis and Treatment of Malaria in line with the World Health Organization's universal diagnostic testing recommendations. MalariaCare, a partnership that provides technical assistance to rapidly scale high quality malaria diagnosis and treatment, is supporting the NMCP to design and implement a malaria case management quality assurance (QA) system with a focus on malaria rapid diagnostic tests (mRDTs). After finalizing an mRDT QA training package, the NMCP and MalariaCare trained regional and district trainers from the Lake Zone, who then trained 1,539 health care workers from 883 public health facilities in May and-June 2015. Following training, MalariaCare and

the NMCP conducted outreach, training and supportive supervision (OTSS) at a subset of facilities in July and September 2015. OTSS aims to reinforce skills developed during the mRDT training and improve adherence to test results through skills observation, mentoring and on-the-spot problem solving. At the first OTSS visit, 197 health workers from 193 facilities scored an average of 93% during mRDT observations, showing a high level of competence post-training. This visit also revealed high average adherence to positive (96%) and negative (91%) test results. Adherence to negative test results varied by facility level, with dispensaries having higher adherence (93%) than health centers (86%) and hospitals (85%). The NMCP and MalariaCare will also present results from the continuation of OTSS, expansion of training and OTSS in the Eastern Zone, and malaria testing rates among these facilities based on HMIS data.

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ALL-IN-ONE, MULTIPLEXED ON-BEAD ELISA FOR MALARIAL BIOMARKERS PLDH AND PFHRPII

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As malaria transmission declines, accurate diagnosis becomes increasingly important for defining disease prevalence and distribution, as well as monitoring impact of interventions. Further, in low-transmission settings, identification and treatment of asymptomatic carriers are critical for eliminating the disease. Current antigen-detecting rapid diagnostic tests (RDTs) for malaria are unreliable in the asymptomatic regime (< 200 parasites/µl), and laboratory protein-based detection strategies, such as well-plate ELISAs, can require 5 - 8 hours of incubation time and are limited to one analyte. To address this, we have developed a multiplexed, magnetic bead-based ELISA for *Plasmodium* lactate dehydrogenase (*p*LDH) and *Plasmodium falciparum* histidine-rich protein II (*Pf*HRPII) with incubation times totaling less than 1 hour and detection limits rivaling those of well-plate ELISAs. In this assay, magnetic particles functionalized with antibodies specific for *p*LDH and *Pf*HRPII are added to parasitized lysed blood samples along with detection antibodies with distinct enzymes for each biomarker. Sandwich complexes for *p*LDH and *Pf*HRPII form on the surface of the magnetic beads, which are washed and sequentially re-suspended in detection enzyme substrate for each antigen. Assay detection limits are 2.7 and 1.2 parasites/µl for *p*LDH and *Pf*HRPII, respectively. Detection of both biomarkers is advantageous because it avoids false-positives due to slow *Pf*HRPII clearance and allows for differentiation between *falciparum* and non-*falciparum* infections, ultimately informing treatment. With these advantages, as well as high sensitivity and detection limits in the single parasite/µl regime, the developed multiplexed assay for *p*LDH and *Pf*HRPII is an attractive alternative to well-plate ELISAs and a promising detection strategy for an elimination setting. Further, the modularity of the multiplexed on-bead ELISA makes it applicable to any series of infectious disease biomarkers for which there are antibody pairs available.

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MALARIA MICROSCOPY COMPETENCY IN LIBERIA POST EBOLA DISEASE OUTBREAK

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Since 2010 Liberia has progressively moved toward parasitological diagnosis of malaria. However in the last two years - since the first confirmed case of Ebola virus disease (EVD) on March 17, 2014, through January 14, 2016, when the World Health Organization (WHO) declared an end to the most recent outbreaks - the public health system has been overwhelmed managing this new disease. Due to risk from blood exposure, national policy mandated returning to clinical diagnosis of malaria instead of drawing blood. Now the Ministry of Health and Social Welfare is in the process of restoring essential and quality-assured health services in governmental and non-governmental health facilities. As a first step, County Health Team Diagnostic Focal Persons (CHT-DFPs) supporting decentralized training, and supervision activities were prioritized for retraining and competency assessment in malaria diagnostics. In February 2016, the National Malaria Control Program, with support from MalariaCare, conducted the first refresher training and microscopy competency assessment for CHT-DFPs from 13 of 15 counties post-the EVD outbreak. Trainees were assessed on parasite detection and parasite quantitation and scored against WHO minimum grades for expert level microscopists. Twelve (12) of 13 participants scored greater than 80% (M 93%; Mdn 94%) on parasite detection and all (100%) participants scored above 50% (M 66%; Mdn 67%) on parasite quantitation, resulting in equivalent designations of WHO Levels 1 (n=12) and 2 (n=1) for these 13 microscopists. There was an 11% improvement between pre- and post-test scores for parasite detection and a 47% improvement was observed for parasite quantitation. Despite an almost two-year interruption in malaria diagnostic services, microscopy capacity within the County Health Teams appears to remain strong. Continued training and monitoring of this cadre using proficiency test panels can be achieved using a recently-procured slide bank, putting Liberia on track to move forward with plans to decentralize training and supervision activities to the county level.

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FIELD EVALUATION OF A REAL TIME LOOP-MEDIATED ISOTHERMAL AMPLIFICATION ASSAY (REALAMP) FOR MALARIA DIAGNOSIS IN CRUZEIRO DO SUL, ACRE, BRAZIL

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Traditional molecular methods, such as nested-polymerase chain reaction (PCR), are very sensitive to detect malaria parasites, but require advanced laboratory equipment and trained personnel. Real-time loop-mediated isothermal amplification (RealAmp), a LAMP-based molecular tool, facilitates rapid target amplification at a single temperature setting, reducing the need for sophisticated equipment. There is limited information on the performance of this method for the malaria diagnosis in clinical settings and field conditions. We evaluated the performance of RealAmp for malaria diagnosis in Cruzeiro do Sul, Acre state, Brazil. We enrolled 1,000 patients with fever (axillary temperature >= 37.5 C) or history of fever in last 24h presenting for malaria diagnosis from February through August 2015. DNA was extracted from dried blood spots using a crude method (heat treatment) at the sample collection site (field site), and using commercial kits at a Brazilian national reference laboratory. Genus-specific RealAmp was performed at both the reference laboratory and field site after appropriate training. In addition, Giemsa-stained blood smears were prepared and examined by two independent well-trained study microscopists. A combination of real-time PCR and nested PCR was used as reference test. The sensitivity and specificity of RealAmp from heat treatment DNA in the field laboratory were 94.1% (95% confidence interval [CI]: 90.1-96.8) and 83.9% (95% CI: 81.1-86.4), respectively, while the sensitivity and specificity of RealAmp at the reference laboratory were 83.2% (95% CI: 77.6-87.9) and 97.0% (95% CI: 95.5-98.0), respectively. Microscopy showed sensitivity of 96.4% (95% CI: 93.0-98.4) and specificity of 98.2% (95% CI: 97.0-99.0). Our findings highlight that it is possible to implement simple molecular tests at point of care in remote areas of countries such as Brazil. However, RealAmp performance was

inferior to that of microscopy performed by skilled professionals. Attempts to develop and evaluate molecular tools should continue, especially in countries targeting pre-elimination.

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CAN PHARMACY PROVIDERS PROVIDE QUALITY MALARIA DIAGNOSTIC IN KENYA: RESULTS FROM EXIT INTERVIEW AND MYSTERY CLIENT STUDIES FROM THE KENYAN COAST

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In Kenya, 32% of people seek treatment in the private sector where availability of diagnostics testing has been low. Pharmacies often serve as the first or only point of accessing care but have not been allowed to perform blood tests, and evidence is required to show that tests can be conducted safely by this cadre. Between 2014 and 2015 PS Kenya implemented a project seeking to increase malaria testing using RDTs among private clinics and pharmacies in Kwale, Mombasa and Kilifi counties.Studies were conducted at a random sample of project facilities in Q4 2014 and Q4 2015 to track provider performance. Exit interviews were held at 130 sites with 526 clients in 2014, and 534 clients in 2015. Eligible cases were adults seeking treatment for fever for themselves or on behalf of someone else. Confirmed RDT-negative volunteers conducted 260 mystery client visits at 155 sites in 2014 and 262 visits at 113 sites in 2015. Data were analyzed using Stata v13. At endline, exit interview clients were more likely to be tested for malaria at clinics than at pharmacies (86.6% vs 59.4%, p<0.001). Between 2014 and 2015 testing by RDT increased by 22.4 points in clinics (30.1% to 52.0%); prior to the intervention RDTs were not formally available at pharmacies. Overall 83% of 238 malaria test-positive clients received an ACT in 2015, with no difference between facility types (p=0.5). However, positive clients at clinics remained twice as likely to receive an antibiotic (46.7% vs 17.1%, p<0.001). Over time untested pharmacy clients were less likely to receive an antimalarial (2014: 40.7%, 2015: 29.4%, p=0.006) while the level at clinics remained unchanged (average: 16%). Mystery clients at pharmacies were more likely to receive the correct diagnosis(negative) (2014: 72.8%, 2015: 79.6%, p<0.001), than in clinics (2014: 58.9%, 2015: 75.7%, p<0.001) At endline, 0% of test-negative clinic clients and 2% of pharmacy clients received any antimalarial, both down from over 7% in 2014.Results from this project show comparable fever management at pharmacies and clinics on the Kenyan coast. To scale up testing the MOH should consider allowing RDT testing at pharmacy-level in Kenya.

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PFHRP2 DETECTING MALARIA RDTS: ALARMING FALSE NEGATIVE RESULTS IN ERITREA

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In Eritrea over 75% of suspected malaria cases are diagnosed using rapid diagnostic tests. Despite following WHO recommended procurement and quality assurance practices, frequent complaints of false negative RDT results were reported from most geographical settings in Eritrea in 2015. Initial investigations involved cross checking RDT results with quality assured microscopy. This exercise confirmed that SD Bioline Malaria Ag Pf/Pv (05FK80) targeting histidine rich protein 2 (HRP2) and *Plasmodium vivax*-plasmodium lactate dehydrogenase (Pv-pLDH), failed to diagnose microscopically confirmed *P. falciparum* malaria, across a range of parasite densities. RDTs were retrieved from the field and found to react against WHO-FIND quality control samples. A product recall was implemented and investigations carried out to delineate causal parasite factors. Specifically, some Pf microscopy positive samples were assessed against other brands of good performing HRP2-detecting RDTs and found to be negative. In February 2016, 50 consecutive microscopically confirmed *P. falciparum* malaria patients presenting at two regional hospitals were screened with non-HRP2 detecting RDTs. Patient specimens returning positive on pf-pLDH test line and negative on HRP2 test line provided blood samples for *Plasmodium* species identification PCR and hrp2/hrp3 PCR. The mean patient age was 29 yrs; parasite density range was 32-89,120 parasites/µl; mean of 15,347). The overall prevalence of falciparum-infected blood specimens with positive RDT results for PfpLDH band but negative to PfHRP2 band was 58% [95% C.I: 44-71]. PCR results for pfhrp2 and/or pfhrp3 gene expression and ELISA for hrp2 are pending but preliminary findings support HRP2/3 gene deletion or variation amongst *P. falciparum* parasites. Furthermore, these findings should be an early warning to neighbouring African countries to follow up reports of false negative RDT results and to consider adding investigations for hrp2/hrp3 gene deletions into surveys and surveillance activities.

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DIRECT, HIGH-THROUGHPUT QUANTIFICATION OF PARASITIC DNA IN MULTIPLE SAMPLE TYPES WITHOUT DNA EXTRACTION

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Accurate diagnosis is essential for successful management of infectious diseases. While most molecular diagnostics are sensitive enough for clinical application, new diagnostic tools with simplified procedure and improved throughput are still needed. Capture and Ligation Probe-PCR (CLIP-PCR) has been demonstrated as a high throughput RNA quantification technology for extreme sensitive identification of malaria, but its role in DNA quantification remains to be demonstrated. In this study, we adopted CLIP-PCR for identification of DNA directly in saliva, buccal swab and whole blood samples. Target DNA from saliva, buccal swab or blood was released by lysis, heat-denatured and captured directly to 96-well plate by sandwich hybridization using multiple oligo probes with universal tail sequences. After enzymatic ligation of the probes, the single-stranded template is quantified with universal primers targeting the tail sequences by qPCR with SYBR green chemistry. To avoid false negatives caused by target polymorphism and reduce turnaround time, we adopted a multi-section strategy: multiple sets of probes that target at a continuous/semi continuous region of targeted DNA were used simultaneously, each of which being sufficient for DNA quantification by CLIP-PCR. Our DNA quantification CLIP-PCR assay was tested for direct quantification of plasmodium 18S rDNA, human 18S rDNA and schistosoma DNA. Without the need for DNA purification, CLIP-PCR quantifies DNA in multiple samples types within 4 hours. *Plasmodium* can be detected at a concentration as low as 0.19 parasites/ul in blood samples. Human 18S rDNA can be detected directly in both saliva and buccal swab samples, while Schistosoma DNA can also also be identified from serum samples of infected mice. These data indicate that CLIP-PCR has the same sensitivity as regular qPCR, but much reduced complexity and cost, making it suitable for large scale molecular surveillance studies.In conclusion, our study showed CLIP-PCR provides a highly sensitive, simple and low-cost means for direct identification of parasitic DNA in multiple sample types in a high throughput fashion.

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HIGH-THROUGHPUT, MULTIPLEX GENOTYPING DIRECTLY FROM SALIVA AND BUCCAL SWABS WITHOUT DNA PURIFICATION

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SNPs have been found associated with disease susceptibility, drug response and complex phenotypes. Clinical population screening of significant SNP markers calls for multiplexed genotyping of SNPs on a large scale. Current SNP genotyping tools, despite many advantages, invariably require DNA extraction, which remains a key throughput-limiting step for population screening. In addition, multiplex-PCR amplification employed by these genotyping methods suffers from complex primer design and/or amplification bias. Here, we describe a novel high-throughput genotyping approach, MELPA , which has multiplex SNP genotyping capability, eliminates DNA extraction, achieves uniform PCR amplification using a single pair of universal primers, and is suitable for saliva and buccal swab samples. In brief, instead of nucleic acid extraction, MELPA lysed saliva/buccal swabs and captured the target DNA directly to 96-well plate by sandwich hybridization using multiple oligo probes with universal tail sequences. After enzymatic extension and ligation of the probes, a single-stranded template for each target SNP site was formed, and all templates were PCR-amplied using universal primers targeting the tail sequences. Multiplexed genotyping by single-base primer extensions were analyzed with a MALDI-TOF mass spectrometry platform. We tested the feasibility of the new assay for saliva and buccal swabs, and evaluated the accuracy by comparing MELPA with commercial multiplex SNP assay (iPLEX), for the detection of 20 G6PD gene variants known to be at risk for primaquine-induced hemolysis in antimalarial therapy. We successfully developed a 20-plex panel for G6PD genotyping. A typical 50 ul saliva or one buccal swab sample is sufficient for running 2 assays. Six 384-samples can be processed from sample to result in a 24-hour workflow, with a hands-on time of 2 hours. Results were consistent with iPLEX, and 100% concordant with sequencing. Saliva and swab samples can be stored at room temperature for at least 24h without affecting the performance. MELPA represents an efficient and cost-effective approach to multiplex SNP genotyping at population level.

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UPTAKE OF MALARIA DIAGNOSTIC TESTS AND ADHERENCE TO NEGATIVE TEST RESULTS AMONG FEVER CARE SEEKERS AT INFORMAL DRUG SHOPS IN UGANDA

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There has been a steep decline in malaria prevalence in Uganda from 43% in 2009 to 19% in 2014. In light of this, the Ministry of Health changed the policy in 2010 to diagnosis prior to treatment for suspected cases to ensure patients are provided appropriate treatment for their illness and to prevent antimalarials being wasted on uninfected patients. Although a third of fever care seeking occurs in the informal private sector, there is scant reliable data on testing rates and appropriate dispensing of antimalarials in that sector. The objective of this study is to provide national estimates for malaria testing rates and to quantify antimalarial misuse. A cross-sectional survey was conducted in March 2016 using two-stage cluster sampling where up to three informal sector drug shops were sampled per cluster. Data were collected using a structured questionnaire on demographics, fever care seeking, and malaria case management. Clients were included if they exited any informal drug shop within the observation period and provided consent. Primary outcomes were proportion of clients seeking care for fever, proportion of fever care seekers testing for malaria by blood slide or malaria rapid diagnostic test (mRDT), and proportion of fever care seekers who tested negative and received an antimalarial. A total of 711 clients from 324 informal drug shops in 124 enumeration areas were interviewed. Seventy percent of all clients sought care at a shop where mRDTs were available. The proportion of clients seeking care for fever was 38%. Among fever care seekers (n=270), 33% were tested at the shop and 11% were tested elsewhere. Ten percent of clients testing negative still received an antimalarial. Among fever care seekers not taking a test (n=153), 60% received an antimalarial. Given the declining trend in prevalence, a sizable proportion of clients with fever are receiving malaria treatment without having malaria, leading to wastage and untreated illness. Educating providers on the decline of malaria prevalence as well as increasing testing availability is necessary to ensure that antimalarials are not presumptively dispensed.

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A MINIATURIZED FLOW CYTOMETRY PLATFORM FOR MALARIA DIAGNOSTICS

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The diagnosis and treatment of malaria remains a challenging global problem due to limitations of highly sensitive and specific tests that identify and type the malarial parasites and due to the long turn-around time and expertise needed for microscopy based tests for determining the degree of red blood cell (RBC) infection. While flow cytometry has been shown to be capable of providing answers in diagnosis of malaria, the traditional use of expensive and sophisticated platforms and highly skilled operators, has restricted the speed and ease with which such diagnostic information can be provided. In this study, we evaluate the potential of a small low-cost, touch screen based miniaturized cytometer, the Muse Cell Analyzer for providing solutions related to malaria diagnostics. The platform is based on microcapillary cytometry, generates low biohazardous waste, uses small sample volume and provides the potential to add multiple diagnostic assays related to global health. A CE/IVD assay for CD4 T cell monitoring was recently released on the platform demonstrating capability of the system for use in resource-constrained settings. We have developed novel multiplexed bead based immunoassay for detection of plasmodium released antigens in blood/serum to enable parasite typing. Initial results demonstrate superior sensitivity for detection of plasmodium antigens to current RDT measurement range. In addition the platform demonstrates capability to provide percentage of infected red blood cells which is also critical to diagnostic decisions. The availability of a simple, easy to use and affordable platform like the Muse Cell Analyzer, that can provide results on plasmodium typing and percentage of infected RBC's can greatly increase capability to provide affordable and timely malaria diagnostics.

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EVALUATION OF THE PARASIGHT PLATFORM FOR RAPID AND HIGHLY ACCURATE MALARIA DIAGNOSIS

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The WHO estimates that nearly 500 million malaria tests are performed annually. While several diagnostic assays are available, the need for an inexpensive, quick and highly sensitive malaria test remains a priority for world-wide malaria treatment. Two recent studies from our group have demonstrated a computer vision platform capable of meeting these needs. Here we present the commercially available version of this technology, the SightDx Parasight platform which provides malaria diagnosis, speciation

and parasite quantification. We conducted studies at Apollo Hospital in Chennai, India where 205 samples were tested, and at Aga Khan University Hospital Nairobi Kenya where 263 samples were evaluated. At both centers the device diagnoses were compared to microscopy, RDT and PCR results. For identification of malaria, the device demonstrated a sensitivity of 99% and a specificity of 100% at Apollo Hospital India, and a sensitivity of 100% and a specificity of 98.9% at Aga Khan University Hospital Kenya. For speciation, the device correctly identified 100% for *Plasmodium vivax* and 100% for *P. falciparum* at Apollo Hospital and 100% *P. vivax* and 99.3% *P. falciparum* at Aga Khan University Hospital. Lastly, comparing the device parasite count with that of a trained microscopist produced an average pearsons correlation of 0.83 at Apollo Hospital and 0.88 at Aga Khan University Hospital.

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NO MORE HIDING: PICOGRAM DETECTION OF HISTIDINE-RICH PROTEIN 2 FROM *PLASMODIUM FALCIPARUM*

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Histidine-rich protein 2 (HRP2) is produced by one of the human malarias, *Plasmodium falciparum*, and is widely used for diagnostic purposes. Detection of HRP2 provides evidence for active or recent infection, but current HRP2 immunoassays are hindered by high detection limits. Here we present a novel HRP2 immunoassay for antigen capture through a bead-based system which is capable of reliable HRP2 detection at low picogram levels in a highly-specific and cost-effective manner. We compared this assay with HRP2-based rapid diagnostic test (RDT) results from community surveys in different *P. falciparum* transmission settings to assess RDT reliability in the general population. In the holoendemic setting of northern Mozambique (RDT: SD Bioline Pf), agreement between the two tests was good, with a kappa coefficient of 0.77 (95% CI: 0.74-0.79). Of 2,280 persons tested, 199 (8.7%) of were found to be positive for the HRP2 protein, but RDT negative, giving a receiver operating characteristic area under the curve (ROC AUC) of 0.95 (0.94-0.96) for the novel test. Additionally, 57 (4.3%) of all RDT positives were found to have no detectable HRP2 by the bead assay, suggesting the RDT false positive rate for this community survey. There was a clear trend for higher HRP2 concentrations in younger age groups and consequent reliability of true-positive RDT results, but this reliability diminished in older individuals. Sampling from the low-endemic nation of Haiti (RDT: First Response HRP2) revealed poor agreement between the tests (kappa: 0.30, 0.14-0.45). Of 4,350 persons tested, 62 (1.4%) were positive by the bead assay, but 53 of these persons were RDT negative (ROC AUC: 0.75, 0.62-0.89), illustrating the low HRP2 concentrations of persons in the population harboring the protein. Of the 24 RDT positive persons from this community survey, 15 (63%) had no detectable HRP2 by the bead assay, showing a high degree of false positive tests in asymptomatic individuals. The low detection limit and high specificity of the bead assay can potentially provide a national malaria control program with an objective indication of the performance of HRP2-based RDTs in an area.

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APTAMER-BASED LOW RESOURCE DIAGNOSTICS FOR DETECTION OF MALARIAL BIOMARKER *PLASMODIUM* LACTATE DEHYDROGENASE

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Early diagnosis of malaria is critical to disease intervention, as asymptomatic individuals with malaria can serve as a transmission reservoir for the disease. Diagnostic testing has doubled since 2010 due to the rise of rapid diagnostic tests, most of which are designed using a lateral flow assay (LFA) format. LFAs overcome many shortcomings of more resource-dependent techniques (i.e. microscopy and PCR) but perform poorly at low parasitemias, thus preventing the diagnosis of asymptomatic individuals. LFAs for malaria often employ monoclonal antibodies (mAbs) for biomarker capture and detection. However, over the past twenty years aptamers have emerged as a potential alternative to mAbs in diagnostic applications. Aptamers are synthetic nucleic acid sequences that bind to target molecules with high (~nM) affinity, and offer several advantages over antibodies, namely a non-immunological origin, increased thermal stability, and affordable automated chemical synthesis. Our lab has characterized the kinetics of binding for a multitude of mAbs and aptamers specific for *P. falciparum* LDH, *P. vivax* LDH, or both. Moreover, we have developed a strategy for capturing native *P. falciparum* LDH from large volume (50-100 µL) whole blood samples using commercial magnetic beads functionalized with an X-aptamer, which is a next generation aptamer that incorporates druglike moieties into various nucleotides of the aptamer sequence to increase target affinity. The captured *p*LDH is subsequently concentrated and eluted in a small volume (10 µL) of single-stranded DNA that is complementary to the X-aptamer, and the eluent is then spotted on a lateral flow assay. This initial processing step overcomes the sample volume constraints (only 5-10 µL) of commercial LFAs, allowing more biomarker to be delivered to the LFA test line for signal enhancement in samples with low parasitemia.

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GUIDING THE DEVELOPMENT OF IMPROVED DIAGNOSTICS FOR MALARIA: LIMIT-OF-DETECTION OF CURRENT RAPID DIAGNOSTIC TESTS

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The simplicity, rapidity and affordability of lateral flow immunochromatographic assays, referred to as rapid diagnostic tests (RDTs), have transformed our ability to diagnose malaria. The performance of quality-assured RDTs is typically equal or superior to routine microscopy and these tests can be used by community health workers effectively and with minimal training requirements. Since 2010, the World Health Organization recommends that all suspected cases of malaria should be diagnosed by either technique to prevent presumptive treatment. As a result, the WHO African Region has seen the proportion of diagnosed cases increasing from 36% of all suspected cases in 2005 to 65% in 2014, with almost three quarters of those being tested by RDTs. This and other improvements in antimalarial interventions led to a global decrease in malaria prevalence with a number of countries shifting from control to elimination strategies.Current RDTs are considered appropriate for the diagnosis of febrile patients, however more sensitive RDTs would be needed to support detection of asymptomatic infections in a context of malaria elimination. In order to guide the development of improved tests, the exact analytical limit-of-detection (LOD) of a set of current RDTs has been determined. The best-in-class RDTs detecting

the histidine-rich protein II (HRP2) or *Plasmodium* lactate dehydrogenase (pLDH) antigens were selected and then tested with serial dilutions of various reference materials, including recombinant HRP2 and pLDH proteins, *Plasmodium falciparum* culture samples as well as field isolates of both *P. falciparum* and *P. vivax*.With this study, we report for the first time the lowest concentrations of target analytes that can be detected by the best RDTs currently in the market. The determination of this key performance parameter allows for a better understanding of the advantages and limitations of current good-quality RDTs as well as of the potential improvement that could be achieved for the detection of parasite populations able to sustain malaria transmission.

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LAMP VERSUS MICROSCOPY AND RDT TO DETECT MALARIA IN PREGNANT WOMEN: A CROSS SECTIONAL STUDY IN NW ETHIOPIA

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Timely diagnosis followed by effective treatment is the best strategy for prevention, control and elimination of malaria in pregnancy. Detection of malaria by microscopy or rapid antigen tests in resource-limited settings is hampered by quality control, infrastructure limitations, training, and poor analytical sensitivity. Low sensitivity of current testing modalities is of concern in pregnancy where parasite levels are low in the peripheral blood due to placental sequestration. We evaluated the performance of loop-mediated isothermal amplification (LAMP) compared to microscopy for the diagnosis of malaria among pregnant mothers in NW Ethiopia. A cross sectional study was conducted from January to April 2016 at Koladiba Health Center in North Gondar. Eight-seven blood samples were collected from pregnant mothers suspected of having malaria and tested by Giemsa-stained thick and thin peripheral bloodfilm, RDT, LAMP and nested PCR. Diagnostic accuracy measures (analytical sensitivity, specificity, predictive values, and Kappa scores) of microscopy, RDT (HRP2/pLDH(Pf/PAN) Combo) and LAMP (Eiken LoopAMP) was compared to nested PCR by using Simple Interactive Statistical Analysis (SISA) software and Cohen's Kappa reliability measure. A total of 87 women were enrolled, 50.6% with a previous history of malaria, 74.7% were multigravidae, 17.2% were in the first trimester, 41.4% second trimester, and 41.4% third trimester. Ten samples were positive for malaria by microscopy, 9 by RDT, and 15 by LAMP. P.falciparum, P.vivax, and mixed infections of the two species were detected. Using nested PCR as gold standard, the sensitivity of microscopy and RDT was 90 and 70%, specificity 98.7% and 97.4%, respectively. LAMP showed a sensitivity and NPV of 100%, specificity of 93.5%, with k=0.768 with nested PCR. We conclude that LAMP is a rapid molecular method and more sensitive than both microscopy and RDT for the detection of malaria in pregnancy. Mass screen and treat strategies to reduce the burden of malaria in pregnancy and reduce infant mortality will benefit from more sensitive methods like LAMP.

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MALARIA STRATIFICATION FOR ELIMINATION ACTIVITIES

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Eliminating malaria and preventing resurgence will require targeting appropriate packages of interventions to places with ongoing transmission while managing the risk of importation and re-establishment, which relies primarily on the capacity of the health system to detect and treat new infections. Here, we describe a process for operational stratification and decision making using three available metrics in the context of a malaria elimination program in Haiti. Maps of malaria incidence data, parasite movement, and treatment seeking rates were generated and used to stratify the country into operationally relevant units. Thresholds for categorizing units by the required intervention packages, including improved routine case detection and management, indoor residual spraying (IRS), active case detection (ACD), mass drug administration (MDA), and measures targeting imported infection, were estimated by analyzing the relationships among the interlinked metrics. This methodology was applied to evaluate interventions required for elimination in Haiti. Results suggested that 75% of the population was at low risk of malaria transmission, requiring only stronger passive case detection and management. Grand Anse in the South West and Commune Ganthier in the West, near Etang Saumatre lake were identified as remote areas with the highest malaria transmission and relatively poor access to malaria treatment, requiring aggressive measures including simultaneous ACD, IRS, and MDA. Most imported infections are found in Port-au Prince suggesting that additional measures targeting travelers would be needed. This methodology provides a potential framework to help national malaria programs to develop evidence-based operational plans for targeting aggressive interventions. In a resource-limited environment, this sort of approach will be critical to ensuring optimal intervention packages are targeted to the places where they will have greatest impact, increasing the probability of interrupting transmission and maintaining malaria elimination.

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INCREASED PREVALENCE OF ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTION IN DIENGA, SOUTHEASTERN GABON

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Control strategies implemented a decade ago led to a marked reduction in the prevalence of malaria in many countries. In Dienga, southeastern Gabon, the prevalence of microscopic *Plasmodium falciparum* infection was 7% in 2003, close to the pre-elimination threshold of 5%. The aim of this work was to determine the prevalence of *P. falciparum* infection in the same community a decade later.A cohort of 370 individuals aged from 3 to 85 years living in Dienga was investigated for *P. falciparum* infection; during six passages (P) in 15-month period. Demographic data were collected, along with behaviors and attitudes towards malaria. *Plasmodium* infection was diagnosed by microscopy (ME), followed by PCR to detect submicroscopic infection.The prevalence of *P. falciparum* infection in P1, P2, P3 , P4 , P5 and P6 was respectively 43.5% (25.1% ME+, 18.4% PCR+); 40.9% (27.0% ME+, 13.9% PCR+), 52.7% (26.1% ME+, 26.6% PCR+); 34.1% (14.1% ME+, 20% PCR+), 57.7% (25.4.% ME+, 32.3% PCR+);and 46.2% (21.4% ME+, 24.8% PCR+) with an overall average of 45.9% (95%CI [37.0 - 54.7], 23.2% ME+ and 22.7% PCR+). P4 and P5 prevalences were statically different throughout the six passages. Microscopic prevalence was significantly higher than that observed ten years ago (23% [n=370] vs 7% [n=323], $p < 0.001$). Asymptomatic infections were the most frequent (96%). Gametocytes were detected in levels ranging from 5.9% to 13.9%. Insecticide-treated nets, indoor residual insecticides, and self-medication were used by respectively 33.2% (95%CI [29.0 - 37.4]), 17.7% (95%CI [15.5 - 19.9]) and 12.1% (95%CI [10.6 - 13.6]) of the study population.A near-threefold increase in *P. falciparum* infection has been observed in a rural area of southeastern Gabon during a 10-year period. Most infections were asymptomatic, but these subjects likely represent a parasite reservoir. These findings call for urgent reinforcement of preventive measures.

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MOLECULAR EVIDENCE OF HIGH RATES OF ASYMPTOMATIC *PLASMODIUM VIVAX* INFECTION AND VERY LOW *P. FALCIPARUM* MALARIA IN BOTSWANA

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Botswana is one of eight SADC countries targeting malaria elimination by 2018. Through upscaling of control activities, significant reductions in case incidence of *Plasmodium falciparum* (0.96 - 0.01) was achieved between 2008 and 2012. As part of the elimination campaign, active detection of asymptomatic *Plasmodium* species was carried out to determine asymptomatic *Plasmodium* species carriage by nested PCR in the country, in 2012. A cross-sectional study involving 3924 apparently healthy participants were screened for *Plasmodium* species in 14 districts (5 endemic: Okavango, Ngami, Tutume, Boteti and Bobirwa; and 9 epidemic: North East, Francistown, Serowe-Palapye, Ghanzi, Kweneng West, Kweneng East, Kgatleng, South East, and Good Hope). Venous blood was taken from each participant for a nested PCR detection of *Plasmodium* species. The parasite rates of asymptomatic *Plasmodium* species detected were as follows: *P. falciparum*, 0.16%; *P. vivax*, 4.66%; *P. malariae*,(Pm) 0.16%; *P. ovale*, 0%, mixed infections (*P. falciparum* and *P. vivax*), 0.055%; and (*P. vivax* and P. *malariae*), 0.027%, (total: 5.062%). The high proportion of asymptomatic reservoir of *P. vivax* was clustered in the East, South Eastern and Central districts of the country. High rates of *P. vivax* infection correlated linearly with high rates of *P. malariae* infections with a predictive value of 27.9 *P. vivax* infections for each *P. malariae* infection (95% CI 22.6-33.2, $p<0.001$). The median age for *P. vivax* infection was 5 years (Mean 5.13 years, interquartile range 3-7 years).The odds of being infected with *P. vivax* decreased by 7% for each year increase in age (OR 0.93, 95%CI 0.87-1.00, $p=0.041$) when gender was adjusted for in a logistic regression. In conclusion, we have confirmed low parasite rate of asymptomatic *Plasmodium* species in Botswana, with the exception of *P. vivax* which was unexpectedly high. This has implication for the elimination campaign, requiring a follow up study taking this evidence into account in the elimination campaign.

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USE OF ACTIVE AND PASSIVE SURVEILLANCE TO DETERMINE THE RISK FACTORS FOR MALARIA INFECTION IN ACEH BESAR, INDONESIA, A LOW-ENDEMIC, MULTI-SPECIES SETTING (*PLASMODIUM KNOWLESI*, *P. VIVAX*, AND *P. FALCIPARUM* INFECTION) AIMING FOR MALARIA ELIMINATION

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As the number of malaria cases declines, the risk factors for infection change and transmission becomes more geographically focal and likely due to asymptomatic and non-falciparum infections. To inform malaria elimination planning, local risk factor assessments using acquired data are necessary. To identify risk factors for malaria infection, a population-based passive and active surveillance study was conducted in Aceh Besar District, Indonesia from 2014 to 2015. Malaria infection was defined as symptomatic PCR-confirmed infection passively reported from five health facilities, or asymptomatic or symptomatic PCR-confirmed infection identified in reactive case detection (RACD). Potential risk factors were assessed through a questionnaire. Multi-level logistic regression models were used to measure the associations between potential risk factors and malaria infection. Risk factors associated with species-specific infection and secondary cases were analysed by chi-squared/Fisher-exact test and Kruskal-Wallis/Wilcoxon test. Passive surveillance identified 19 *Plasmodium knowlesi*, 11 *P. vivax*, and six *P. falciparum* infections. Of 1,495 individuals screened in RACD, six (one Pk, three Pv, and two Pf) had PCR-confirmed infection. Compared to non-infected subjects screened in RACD, infections identified through passive or active surveillance were more likely to be male (AOR 12.24, 95%CI:2.84-51.05), young adults (15-30 years) (AOR 14.11, 95%CI:2.01-98.79), and work requiring overnight stays in the forest (AOR 8.19, 95%CI:1.54-43.70). Clustering of species by sub-district of residence was determined. For secondary case detection in RACD, cases were mainly afebrile (4/6), resided within 100 meters or in the same household as the index case, and had the same risk factors as index cases. The risk factors for infection in index cases and RACD identified cases were related to forest-related work. In low transmission settings, utilization of data available through routine passive and active surveillance can support targeted efforts for individuals at high risk.

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USE OF 'EASY ACCESS GROUPS' TO GUIDE MALARIA ELIMINATION INTERVENTIONS IN HAITI - SURVEYS AMONG PRIMARY SCHOOL, HEALTH FACILITY AND CHURCH ATTENDEES TO REFINE TRANSMISSION RISK AREAS AND TARGET INTERVENTIONS

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"Malaria Zero: the Alliance for a Malaria-Free Haiti" was formed to assist Haiti in achieving malaria elimination by 2020. *Plasmodium falciparum* prevalence is estimated to be <1% nationally. In such low transmission settings, infections are likely to be spatially clustered. Targeting elimination activities to these clusters rather than the whole population will be a more effective use of resources and is expected to have a greater impact on transmission. This study investigates the use of surveys among easy access groups (EAG) to identify areas of ongoing transmission where aggressive parasite elimination activities can then be focused. Three EAG venues were identified to enable rapid sampling of populations: primary schools, health facilities (all attendees) and churches. A concurrent large-scale household survey was conducted to validate which, if any, EAG sampling approach(es) can identify areas of ongoing transmission in the community to support decision making for targeting elimination activities. EAG surveys were conducted in 25 primary schools, eight churches and 10 health facilities across four communes of Grande Anse, with 2,100 individuals randomly sampled from each venue type. Each individual was tested for a *P. falciparum* infection using an HRP2-based rapid diagnostic test (RDT) and exposure to malaria by screening for a panel of antimalarial antibodies by Luminex. Data on demographic and socio-economic factors, treatment seeking behavior, travel patterns, and prevention practices were also collected. Information on residence was collected from all RDT positive and a random selection of RDT negative individuals using a variety of approaches (GPS loggers, interactive digital maps) compared with direct tracing of households. Residence information is used to estimate the approximate catchment area for each EAG venue, as well as locate the household of detected *P. falciparum* infections. Data collected from the household survey will be used to calibrate EAG data, and determine if EAG surveys successfully identified areas of ongoing malaria transmission.

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PRIMAQUINE SAFETY IN G6PD-DEFICIENT MILITARY COHORT IN CAMBODIA USING THE LOWER-DOSE, EXTENDED COURSE REGIMEN AS PART OF MASS DRUG ADMINISTRATION FOR MALARIA ELIMINATION

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Primaquine (PQ) implementation in malaria-endemic areas is hindered by the fear of precipitating primaquine induced hemolytic anemia in G6PD-deficient subjects. We report on safety of a modified dosing regimen of primaquine in G6PD-deficient subjects, with G6PD activity ranging from 0.04 to 2.67 U/g Hb (median 0.59 U/g Hb) who got treated with a lower dose, extended 12 week course of primaquine as part of the Malaria Elimination Pilot Study in Cambodia. This dose of PQ was well tolerated even in subjects with severe G6PD deficiency and appears to carry a low risk of significant hemolysis. 87 of G6PD-deficient volunteers were started on a weekly PQ at a dose of 22.5 mg (approximately 0.45 mg/kg) for 12 weeks, for a total dose of 270 mg. All subjects who had >10% drop of HCT and/or Hgb on day 3 post PQ, had additional CBC performed on day 7 with safety follow up until their counts stabilized. Thirteen of 87 (15%) G6PD-deficient volunteers, treated under the modified PQ regimen, had more than 10% drop in HCT and/or Hgb on day 3, the highest drop being 16%. On day 7, 2/13 had no change, 2/13 showed recovery (rise in HCT and less than 10% down from baseline), and 9/13 showed continued HCT reductions in the range of 13-22% from baseline. 3/13 volunteers had additional small drops of HCT in week 2 but none exceeded 25% safety threshold or required PQ discontinuation. The lowest Hgb value measured post PQ was 11 g/dL. No volunteers had serious adverse event due to PQ or significant symptoms of anemia or hemolysis. This is the largest cohort of G6PD-deficient subjects from Southeast Asia treated with weekly PQ and followed prospectively. These results contrast recent

report of significant safety concerns with the CDC-recommended 45 mg weekly PQ regimen in G6PD-deficient subjects in SE Asia. The better safety profile of this lower 22.5 mg weekly dose offers a more feasible strategy for targeting hypnozoites in malaria elimination settings where *P. vivax* and G6PD deficiency are common.

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COMPARATIVE ACCURACIES OF THREE MODELS OF HOTSPOT PREDICTION IN THE PRE-ELIMINATION SETTING OF ZAMBEZI REGION, NAMIBIA

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As malaria transmission in pre-elimination settings is geographically heterogeneous, successful programs will target hotspots efficiently. These are foci with transmission intensity greater than that of surrounding areas, inside which parasite reservoirs can persist. Programs often use historical health facility (HF) case data to predict hotspots, though these are coarse data and can miss new hotspots. We compare the predictive abilities of a model utilizing solely historical HF case data, to models incorporating prevalence and/or serologic measures. The study was conducted in the catchment areas (tot. pop.~35381) of 6 randomly selected HFs in Zambezi region, Namibia, a low transmission eliminating nation. The region's annual incidence of 26/1000 contrasts with 2/1000 nationally. Potential hotspots were identified as cases clustered in space/time using SaTScan™. The datasets were: (i) HF case data extracted from registries for Jan 2012 - May 2014; (ii) PCR-confirmed parasite prevalence in a 2015 cross-sectional survey; (iii) seropositivity to AMA-1 and MSP-1 antigens in the cross-sectional survey. Receiver operating characteristic (ROC) curves are generated for clusters identified by each method. The reference value is confirmed hotspots: villages with ≥2 laboratory confirmed, locally acquired cases reported in the 2015-16 season, with geo-location confirmation. A model based solely on HF incidence (1914 total cases) revealed 2 clusters of 58 and 36 cases with radii 3.2km and 4.4km and relative risks 2.04 and 2.33, respectively ($p<0.001$). A model using prevalence (25/2017, 1.2%) demonstrated no statistically significant clusters. Serologic assays are complete and undergoing quality control. Final analyses of dataset combinations with ROC comparison are anticipated before May 30. If a hotspot prediction model including serologic data is more accurate than those using only historical incidence and/or point parasite prevalence, serologically defined hot spots may represent a useful surveillance tool in Zambezi region. Evaluation of an even broader array of candidate antibody responses can help further refine the model.

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ENTOMOLOGICAL MONITORING ACTIVITIES DURING A MALARIA ELIMINATION PILOT PROJECT IN SOUTHERN MOZAMBIQUE

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The National Malaria Control Programme (NMCP) of Mozambique, in collaboration with key in-country malaria control stakeholders under the umbrella of the Mozambican alliance towards the elimination of malaria in Mozambique (MALTEM) and the Mozambique, South Africa, Swaziland (MOSASWA) Cross-Border Malaria Initiative, is currently designing and piloting activities to eliminate malaria from southern Mozambique by 2020. Here we show the first data from ongoing entomological monitoring in Magude, a rural district in the southern Mozambique, where we carry out a pilot malaria elimination project. We address critical issues such as (i) the anopheline species present and their vectorial capacity, (ii) how species distribution and intensities change over space and time, and (iii) which species act as a reservoir of parasites during the dry season. In addition, changes in species distribution, densities and behavior can be observed as a result of vector control interventions.

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TIMED AND TARGETED MALARIA TESTING DURING LOW SEASONAL MALARIA TRANSMISSION IN LUAPULA PROVINCE AS A POTENTIAL STRATEGY TOWARDS ACHIEVING MALARIA ELIMINATION IN ZAMBIA

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According to the Zambia National Malaria Control Centre (NMCC), about 98% of malaria cases are caused by *Plasmodium falciparum*. From May to August, malaria transmission is at its lowest level partly because female *Anopheles* mosquitos are in aestivation, and malaria vector population is reduced. To contribute to Zambia's health strategic plan of eliminating malaria by 2020, World Vision implemented the Stop Malaria Project in 11 districts of Luapula province (Kawambwa, Nchelenge, Mansa, Samfya, Chiyengi, Mwense, Chembe, Mwansabobwe, Chipili, Lunga and Milenge) from April 2014 to March 2016 and distributed 1,172,790 long-lasting insecticidal nets (LLINs) to over 2 million people. Reducing parasite prevalence in an intermediate host is an important step in malaria elimination. As the use of rapid diagnostic tests (RDTs) are the most recommended way of detecting malaria in high incidence areas and in low-resources settings with limited malaria microscopy services, the timed and targeted malaria testing strategy was used to identify parasite prevalence. Two approaches for detecting malaria carriers were used: a) testing household members who received a LLIN after mass distribution and b) testing household members where a malaria patient came from. Malaria parasite detection increased about three times through index case testing or active case surveillance (1,540 per 1000 people) compared to households where random testing was done (464 per 1000 people). Through both approaches, a total of 24,937 were tested for malaria (8,735 people tested through index case testing and 16,202 people randomly tested) and 8,393 were positive (4,570 and 3,823 people tested positive respectively). The malaria timed and targeted testing strategy using the index case approach during low transmission helps to increase detection of malaria parasites in human reservoirs. It should be used during vector aestivation and should be supported by proven vector control interventions that can further reduce malaria transmission by *Anopheles* mosquitoes.

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ASSESSING ASSOCIATIONS BETWEEN RECENT TRAVEL AND MALARIA PARASITE PREVALENCE DURING A MASS DRUG ADMINISTRATION CAMPAIGN IN SOUTHERN ZAMBIA

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Southern Province, Zambia has seen substantial reductions in malaria incidence over the past few years; as malaria incidence approaches zero, challenges remain in preventing importation from neighboring areas of higher transmission. The effect of human mobility on malaria incidence is unknown in this area of Zambia and efforts to identify high-risk scenarios for importation are needed. During a mass drug administration campaign conducted in diverse malaria settings in Southern Province from late 2014 to early 2015, participants were tested for *Plasmodium falciparum* using rapid diagnostic tests (RDTs) and asked about recent travel away from their place of residence. Of those that travelled in the past 14 days, both the origin and destination of each trip were categorized as low or high transmission according to area-specific malaria incidence rates (10% IR threshold). In total 2,862 (0.9%) reported traveling at least once during the 2-week period prior to the campaign visit, of which 762 were to high transmission areas; these travelers accounted for 270 (1.4%) identified infections. After adjusting for confounding factors such as vector control, age, sex and malaria incidence around their home, logistic regression showed that those traveling were twice as likely to be infected [adjusted odds ratio (AOR) = 2.3, 95% Confidence Interval (CI) 1.9-2.6] compared to those who had not traveled recently. Each day of travel conferred an average 6.4% (95% CI: 3.3-9.4%) increase in the odds of RDT positivity. Parasite prevalence was 4.9 and 9.5 times higher among participants traveling to high transmission areas for 3 or less days and 4 or more days, respectively, compared to those traveling to areas of low transmission. The increased malaria prevalence among those traveling, especially to high incidence areas, as well as the incremental increase in the odds of infection per day of travel, suggests that importation back to low incidence areas is a potential concern for elimination efforts and, though currently only a marginal contribution to overall incidence within this region, will be import to reassess as malaria programs approach elimination goals.

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DEMOGRAPHICS AND MALARIA PREVENTION IN MOBILE AND MIGRANT POPULATIONS IN SOUTHERN LAO PDR

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Lao PDR is committed to malaria elimination by 2030. Although transmission is endemic, 97% of confirmed cases are reported from the 5 southernmost provinces. Mobile and migrant populations (MMPs) in these provinces are considered to be at risk for malaria and may be missed by surveillance systems. Appropriately targeted interventions are constrained by a shortage of information on MMP demographic, travel and health patterns. The objective was to improve understanding of these populations by describing demographics, movement, labor practices, and malaria prevention among MMPs in southern Lao PDR. Participants were confirmed malaria cases recruited from health facilities between February 25 and March 25 2015 and were eligible if they had moved from their residence within the past 12 months to stay or work elsewhere. Family and work contacts identified by enrolled cases were also invited to participate. The resulting sample population (MMPs) included all initially enrolled cases and contacts. Information on MMPs was collected via semi-structured face-to-face or telephone interviews. Descriptive statistics and logistic regression were used to characterize MMPs and compare malaria prevention methods between occupational groups. Of 189 MMPs, 69% were male. While most MMPs (78%) reported a province within Lao PDR as their place of origin, 20% were from Vietnam and 2% from other countries. Three quarters (76%) of MMPs traveled with at least one immediate family member. While 95% reported sleeping under a net or hammock the night prior to being surveyed, 82% described the net or hammock as untreated. Twenty-six percent of construction workers slept under treated nets compared with 4% of logging workers. Odds of sleeping under treated nets increased with family members present (OR=1.3 for each additional family member; p=0.033). Demographic differences and variation in malaria prevention behaviour observed in this study may indicate sociological patterns that could inform future targeted long-lasting insecticide-treated net or hammock campaigns among MMPs in southern Lao PDR.

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AN INVESTMENT CASE FOR MALARIA ELIMINATION IN THE PHILIPPINES

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The Philippines has made significant progress in malaria control, with an 83% reduction in cases and 89% reduction in deaths since 2005. The country has launched a subnational approach towards malaria elimination and aims to reach countrywide elimination by 2030. To attain this goal, the National Malaria Control Program (NMCP) of the national Department of Health must secure sufficient funding and political commitment amid waning focus on malaria and declining donor funding for malaria-eliminating countries, including the Philippines. To support advocacy and resource mobilization efforts of the NMCP, we developed an investment case for malaria elimination and prevention of reintroduction (POR) in the Philippines. For this, we estimated the costs of malaria elimination and POR efforts in the Philippines for year 2015 using a micro-costing approach and projected the costs of eliminating malaria in the next 5 years (2016-2020). We estimated the economic benefits of investing in malaria under a hypothetical scenario of resurgence that would likely occur if all efforts on malaria are halted. The benefit of sustained investments in malaria was estimated based on the cost and impact of a potential malaria resurgence on the health system, households, and economic productivity. Cost data were collected from the NMCP and from five sample districts that were in varying phases of elimination. Our preliminary results indicate the total cost of elimination and POR activities in the Philippines in 2015 was US$ 1.03 per capita. The key cost drivers were consumables (61% of total cost) and human resources (21% of total cost). Among malaria activities, prevention and vector control incurred the highest share of the total cost (42%), followed by diagnosis (24%) and program management (15%). The benefits of investing in malaria under the hypothetical resurgence scenario were estimated to be around US$1 billion yielding a return on investment of 8.19. This remarkable return on investment in malaria makes a compelling case for sustaining investments for malaria elimination efforts in the Philippines.

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REACTIVE CASE DETECTION FOR MALARIA ELIMINATION

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The transmission mechanism of many infectious diseases results in spatiotemporal clustering of cases. In near-elimination scenarios we can take advantage of this structure, by using the passive detection of an index case as the basis for active searches for further cases. This strategy, known as reactive case detection, is particularly attractive for moving towards elimination in settings where effective universal surveillance is not feasible. We report on microsimulations and analytical models for *Plasmodium falciparum* malaria, based on epidemiological and programmatic data from elimination projects in the Lake Kariba region of southern Zambia, which we use to quantify thresholds for success for realistic implementations in terms of transmission context, case-enrichment ratios and health system capacities. Mass drug administration has been proposed as a useful adjunct intervention to elimination programs, and we use our models to establish the contexts where this is likely to have a significant effect on the outcome and timeline of the overall strategy. The models provide an insight into the limits and potential of such strategies, which is applicable in other malarial regions and indeed in other disease areas where the focus has moved from control towards elimination.

902

MALARIA ELIMINATION IN INDIA: LARVIVOROUS FISH PLAY AN IMPORTANT ROLE UNDER LARVAL SOURCE MANAGEMENT STRATEGY

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National Framework for Malaria Elimination in India was launched on 11 February 2016. The aim is to eliminate malaria i.e. zero indigenous malaria transmission in the entire country by 2030. Based on annual parasite incidence (<1 case per 1000 population/year), the country has been classified in to three categories. Karnataka state, south India falls under category 2. Karnataka was endemic for malaria till 2009. Currently, except two coastal cities malaria in rural areas has attained pre-elimination phase. The fish-based malaria control was initiated in the early 1990's. The main compulsion of this strategy was scientifically adopted in sericulture areas in the state from 1994. Seeing this result this method of malaria control was extended in the state. Two self-sustained larvivorous fish - mosquito fish *Gambusia affinis* in ponds and tanks, while guppies in open-dug wells formally reduced the main vector *Anopheles culicifacies* population. In most of the rural areas malaria has reached a phase which needs to prevent the residual transmission possibilities. Efforts are being made to mitigate the elimination challenge in the state and aim to eliminate malaria before by 2022.

903

EVALUATING STRATIFIED MALARIA CONTROL INTERVENTIONS IN BIOKO ISLAND: DIFFERENT APPROACHES TO FOCALIZED INTENSIFIED MALARIA CONTROL INTERVENTIONS THROUGH SPATIAL CLUSTERING AND RISK MAPS

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The Bioko Island Malaria Control Project (BIMCP) created a geo-referenced mapping system in 2012 assigning a unique identifier to all households similar to an address. In 2014 this mapping system, based on ArcGIS software and satellite imagery, was linked to the Campaign Management Information System (CIMS), an Android-based tablet application, and is currently used to plan, implement, and monitor field malaria control activities on Bioko; ultimately allowing the BIMCP to accurately track all malaria control interventions at the household and community level over time. To account for budget constrains essential for long term-sustainability of malaria control programs, a stratified control strategy can provide a possible sustainable and reproducible solution. In 2015, the BIMCP developed a framework for Indoor Residual Spraying (IRS) using a stratified methodology to target communities with higher risk of malaria prevalence. The model used: pre-existing Malaria Indicator Survey (MIS) data focusing on prevalence and risk of importation, housing characteristics for all households, spray coverage, and slope and altitude. Information is linked to the unique household identifier and a risk score is created at the community level identifying the most vulnerable communities that would be selected for IRS. Using this pre-existing model for stratification, additional analysis will be carried out focusing on two different spatial clustering techniques: 1) Kulldorff's spatial scan statistics using SaTScan v9.4.2 and 2) Anselin's Local Moran's I statistics using ArcGIS v10.4; as well as regression analysis including data on socio-economic status, bed net coverage, and malaria incidence. Due to the unique characteristics of the methodologies that will be used for stratification, the parameters will not be analogous, but various thresholds will be taken into consideration in order to achieve a higher degree of comparability. Once all models have been completed and quantitatively verified, maps will be created for each methodology, overlaying the results into one map, in order to provide evidence of visual clustering of malaria risk areas.

904

THE USE OF SCHOOL RECORDS TO MEASURE THE IMPACT OF A MALARIA CONTROL INTERVENTION ON ATTENDANCE IN RURAL WESTERN KENYA

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Malaria control in school-age children is an important priority in endemic countries due to the detrimental effect of malaria on academic performance and school attendance. Additionally, the high frequency of malaria infection and gametocytemia in this age-group may contribute substantially to transmission. We reviewed public primary school attendance records of children living in villages in Siaya County Kenya that had been in enrolled in a cluster randomized trial (CRT) of a malaria intervention in 2010. The CRT showed a 42% reduction in the incidence of malaria infection in children ages 5-11 years who lived in

the intervention villages compared with those in control villages. We assessed attendance records from 2010 at 7 schools serving 8 of the 12 villages in the CRT for evidence of spillover effects from the home-based intervention. There were no records available for 2010 in 4 additional area schools. We evaluated school records for data quality by the four domains of the Prism tool: completeness, accuracy, timeliness, and relevance. We used several methodological approaches to assess attendance: time series analysis, mixed effects, and GEE, with and without imputation to adjust for missing data. Attendance records on 914 children were included in the analysis. We noted 43.7% missing data; evidence of over writing and post-date marking of registers; sample means higher than national and local mean by head count. Data quality was not adequate in any of the four Prism domains. Attendance was higher in the treated versus control group ranging from 0.67% to 0.96% (SE 0.3%, $p<0.05$), but this finding was not retained following imputation. We were unable to show an increase in school attendance associated with an intervention that reduced malaria incidence. The lack of detectable effect from the intervention may be due to asymptomatic parasitemia in this age-group, or bias from missing and poor quality data. School attendance records are not a reliable source of observational data in developing countries. Routine records should be fully vetted using a quality framework before being used as a data source document to evaluate interventions.

905

TREATMENT ADHERENCE TO DIHYDROARTEMISININ-PIPERAQUINE DURING MASS DRUG ADMINISTRATION FOR MALARIA IN SOUTHERN PROVINCE, ZAMBIA

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The effectiveness of the mass drug administration (MDA) to prevent and control malaria transmission is, in part, dependent upon the patient's adherence to the medication regimen and achieving high population treatment coverage rates. In late 2014 in Southern Province, the National Malaria Control Center of Zambia embarked upon a community randomized controlled trial of two MDA strategies with dihydroartemisinin-piperaquine (DHAp): focal MDA (fMDA), where treatment was provided to all household members if at least one household member tested positive; and, community-wide MDA, where treatment was provided to all people irrespective of test positivity. Study participants were tested for malaria, provided three doses of DHAp if treatment eligible, and visited on the third day for follow-up monitoring. The first dose of DHAp was directly observed. Analysis from the first two rounds among 75,376 persons treated indicate that 84.6% were fully adherent by reporting taking three doses of DHAp and had blister-pack confirmation and 2.9% reported taking no doses. The proportion of full adherence was greater in fMDA (91%) than MDA (81%) ($p<0.01$). The odds of full adherence in fMDA were more than two times than that in MDA, when controlling for individual RDT status and whether first dose was observed (OR 2.39, 95% CI 1.26-4.53, n=73,703). Reasons provided for incomplete adherence were forgot doses (13.6%), felt better (13.2%) and lost medication (7.4%). These results suggest that individuals recognized that if they their household qualified for treatment, they were either at risk for malaria and/or sought to clear their infection. MDA treatment campaigns may enhance treatment adherence by assuring direct observation of the first dose and sensitization efforts in areas where non-RDT positive individuals are provided treatment. Results will be updated with additional treatment rounds and expanded with comparison to household malaria indicator survey data.

906

ACCELERATING THE REDUCTION OF MALARIA TRANSMISSION IN KANEL, RANÉROU AND LINGUÈRE DISTRICTS (SENEGAL): CASE INVESTIGATION WITH FOCAL DRUG ADMINISTRATION

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Systematic investigation of malaria cases and neighboring households is a key strategy in the path to elimination in low malaria transmission settings. A pilot quasi-experimental study to evaluate whether case investigation with focal drug administration (FDA) can decrease malaria incidence was conducted during the 2015 transmission season in six health post catchment areas in the districts of Kanel, Linguère, and Ranérou (Senegal) that had received a mass test and treat campaign in 2014. Seven adjacent health posts with similar characteristics but lower malaria incidence were selected as comparison. Malaria cases passively diagnosed at the health posts or by community health workers were visited at home and all members of the household were tested with a rapid diagnostic test (RDT) and treated with dihydroartemisinin-piperaquine (DHAP). The closest five households in a 100 meter radius were also visited, all members were tested with an RDT, and in households with at least one positive RDT, all members were treated with DHAP. From September to December 2015, 1560 malaria cases in catchment area residents were passively detected (57% were male, 9% were <5 years old and 60% were aged 5-19 years old). Among these cases, 794 (51%) were investigated and 1887 households were visited, in which >95% of the members were tested. Among the 766 non-investigated cases, 71% recently received the intervention through the investigation of another case or an FDA conducted in response to an outbreak. The RDT positivity rate in the case households was 4.4% (285/6480), whereas in neighboring households it was 3.5% (250/7053). Treated individuals received a follow-up visit a median of 3 days after the initial visit; compliance with the 3-day treatment was high (>95%) and all adverse events were mild. To evaluate the impact of the interventions, the incidence of passively detected, RDT-confirmed malaria cases at the health posts will be compared before and after the intervention and between intervention and comparison villages using a difference-in-differences analysis. Results from the full transmission season and the impact evaluation will be available mid-2016.

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HETEROGENEOUS PREVALENCE OF SUBCLINICAL MALARIA MEASURED BY ULTRASENSITIVE PCR IN MYANMAR

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A malaria elimination campaign underway in the Greater Mekong Sub-region (GMS) aims to prevent the spread of artemisinin-resistant falciparum malaria beyond the region. Elimination may require drug treatment of all malaria infections, including low density, subclinical infections that may represent a previously unrecognized transmission reservoir. In Myanmar, targeted mass drug treatment is being evaluated using ultrasensitive PCR-based testing that is thousands-fold more sensitive than rapid diagnostic tests (RDT) and hundreds-fold more sensitive than standard PCR. In collaboration with seven governmental and non-governmental malaria elimination partners, we conducted cross-sectional surveys of malaria prevalence in 43 villages located in 13 malaria endemic rural townships of in nine State and Regions of Myanmar. Finger-prick blood was collected for rapid diagnostic testing and for standard PCR and ultrasensitive multiplex reverse transcription real-time PCR (usPCR) analyses. In preliminary analyses, *P. falciparum* prevalence (both mono-infection and mixed with *P. vivax*) ranged from 0-10% by RDT, and 0-30% by usPCR; and *P. vivax* (both mono- and mixed with *P. falciparum*) 0-3% by RDT and 0-28% by usPCR. Prevalence by standard PCR, regression analysis of the data adjusted for covariates, and comprehensive village-level geospatial mapping of malaria prevalence will be presented. Subclinical malaria at very low densities can be reliably detected by a new, DNA and RNA-based, fingerstick usPCR method. The prevalence of malaria in Myanmar is highly heterogeneous from village to village, even within the same township, highlighting the need for microstratification of malaria risk to target interventions. Prospective longitudinal studies assessing the clinical and transmission risks posed by this subclinical malaria reservoir are being planned. Results are expected to guide decisions about whether, when, where and how to implement targeted mass treatment and other interventions to eliminate this reservoir.

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REACTIVE CASE INVESTIGATION WITH REACTIVE FOCAL TESTING AND TREATMENT FOR MALARIA IN TARGETED REGIONS IN ETHIOPIA AND SENEGAL: OPERATIONAL LEARNINGS

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In low malaria transmission areas, case investigation—of individual malaria cases, their household, and neighboring households is an important tool to contain and prevent the spread of transmission. Case investigation for malaria was implemented in six health post catchment areas in Kanel, Linguère, and Ranérou (Senegal) and ten villages in Amhara Region (Ethiopia) during the 2014 and 2015 transmission seasons. When a malaria case was passively detected at the health post or by a community health worker, it was considered an index case and a field team started an investigation targeting the case's household and the closest neighboring households (up to 5 households in Senegal and 10 in Ethiopia) within a 100-meter radius. All household members were tested with a rapid diagnostic test (RDT) and treated with an antimalarial drug if positive. Operational indicators were calculated to inform the planning and training and to understand the field teams' compliance with the standard operating procedures. The mean number of households visited per investigation by health post catchment area ranged from 1.9 to 3.1 in Senegal and 1.4 to 6.4 in Ethiopia. In Senegal, the mean distance between the index case household and the neighboring households was of 77.29 meters and that between the index case household and neighboring households with positive RDTs was of 47.5 meters. The following additional indicators will be calculated when final data are available: the average number of households existing within 100 meters of an index case household, the percentage of these that were visited by the field teams during the investigations, the percentage of visited households that were within 100 meters of the index case household, and the average distance to households that were visited beyond the 100 meters radius. The relationship between distance from index case household and RDT-positivity rate will also be evaluated. Final results using complete data from both countries will be available by mid-2016.

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DO DASHBOARDS MATTER TO DISTRICT HEALTH MANAGERS? DOCUMENTED EXPERIENCE FROM THE DEVELOPMENT AND TESTING OF VISUALIZATIONS, DASHBOARDS AND ALERTS FOR MALARIA ELIMINATION IN SOUTHERN PROVINCE, ZAMBIA

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As more National Malaria Control Programs focus on malaria elimination, real time, accurate, and actionable data are critical to target interventions to specific geographies and populations and to optimize the allocation of resources. With decentralization of decision making in Ministries of Health across Sub-Saharan Africa, decision making power will be in the hands of district health and facility managers. Efforts to date have focused on collecting data and ensuring data quality so district managers can

manage scarce resources effectively. Questions remain as to what format and frequency should these data be available to district managers and how to use the data to facilitate stronger analysis and action especially at the district level. As the Zambian Ministry of Health is embarking on an ambitious effort to eliminate malaria with significant investment in the surveillance infrastructure and data quality, we examined different visualization, dashboard and alert mechanisms tailored for district health managers and community health worker cadres. Our user group assessments were conducted in 15 districts in Southern and Lusaka Provinces. District health manager input was used to co-design and develop dashboards to facilitate better planning and action including dashboards assessing reporting, data quality, malaria case rates, case investigation and commodity stocks. We also developed and tested the usefulness of different alert systems using SMS, email and web-based communication. This co-development approach, produced well-documented promising practices on how to create and test dashboards that can help or hinder decision making for district health managers. The process of producing dashboards provided further insights into optimizing visualizations and focusing training of country counterparts. In sum, joint development of data visualization appears to improve data use for decision making.

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AUDIT OF MALARIA DEATHS REPORTED IN THE ROUTINE MALARIA INFORMATION SYSTEM (RMIS) IN FOUR REGIONAL DEPARTMENTS, BENIN, 2015

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Malaria is the leading cause of mortality in children <5 years in Benin. National guidelines on reporting malaria death were established in 2010. Despite recent improvements in malaria case management and case reporting, the annual number of malaria specific deaths (MSD) – 1,869 in 2014 – remains high. To investigate Benin's MSD burden, the National Malaria Control Program conducted a cross-sectional study to examine case reporting and the quality of care for severe malaria cases. Using patient health records from 24 health facilities reporting at least 10 malaria deaths in 2014, we randomly sampled 20% of malaria deaths captured by the routine malaria information system and verified diagnosis, cause of death, and treatment consistency with national guidelines. A case was confirmed an MSD if the record included time and cause of death, confirmed malaria diagnosis by microscopy or rapid diagnostic test, and documented at least one sign of severe malaria. Quality of care was assessed based on: diagnostic confirmation of malaria, injection of the recommended intravenous antimalarial at the right dosage, and correct case management according to clinical signs of severity. We identified 294 records reporting malaria deaths. Of these, 210 (71%) were correctly reported as MSD. None of the health facilities surveyed had a copy of national guidelines on defining MSD. Among the 210 MSD, we were able to assess quality of care in 204. Of these, 151 (74%) deaths occurred in the first 24 hours of care and 186 (88%) were < 5 years of age. Of 204 MSD with signs of severe malaria, 190 (93%) received intravenous antimalarials, but only 34 (17%) received treatment that followed national guidelines. Anemia and convulsions were the most reported severe signs, occurring in 125 (61%) and 97 (48%) of cases, respectively. Seventeen anemia cases (14%) received a blood transfusion. Ten convulsions cases (10%) received diazepam as indicated. Poor knowledge of national reporting guidelines may influence the high number of reported malaria-specific deaths in Benin, however poor adherence to severe malaria treatment guidelines likely contributes to high malaria mortality as well.

911

EVALUATING THE COVERAGE AND IMPACT OF A UNIVERSAL COVERAGE BED NET CAMPAIGN IN TWO DISTRICTS IN NAMPULA PROVINCE, MOZAMBIQUE: A SERIES OF CROSS-SECTIONAL HOUSEHOLD SURVEYS

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Malaria is a principal cause of morbidity and mortality among children in Mozambique, with the northern provinces among the highest-burden areas in the world. One of the chief strategies to reduce this burden is the mass distribution and promotion of long-lasting insecticidal nets (LLINs). In 2013, the Ministry of Health implemented a universal coverage distribution of LLINs in highly endemic Nampula Province. An evaluation was commissioned to measure the coverage and impact of the campaign. Two cross-sectional household surveys, two weeks after the distribution in 2013 and one year later, were performed in Nacala-a-Velha and Mecubúri Districts of Nampula. Households chosen using two-stage cluster sampling were visited by survey teams. Surveyors interviewed the head of household, directly observed nets and sleeping spaces, and performed malaria rapid diagnostic tests (RDTs) on all household members, regardless of symptoms. Indicators of LLIN ownership, access, and use were calculated, and malaria RDT positivity in children under five was compared between years. A total of 1,027 households visits were made, with 2,419 individuals tested by RDT. In Nacala-a-Velha, 80% (95%CI: 72-86) of households received at least one LLIN, corresponding to 66% (58-74) of sleeping spaces. In Mecubúri, 54% (44-65) of households received at least one LLIN and 43% (35-52) of sleeping spaces were covered. The proportion of the population that reported using LLINs more than 4 times per week during the wet season was 43% (29-58) in Nacala-a-Velha and 26% (20-33) Mecubúri, falling to 22% (15-30) and 18% (14-23), respectively, during the dry season. Malaria RDT positivity in children under five was 52% (36-67) in 2013 and 61% (44-76) in 2014 in Nacala-a-Velha and 67% (53-80) in 2013 and 87% (76-94) in 2014 in Mecubúri. In the two districts, the government-led campaign did not reach the crucial coverage and usage threshold to result in a reduction in malaria positivity. The results reinforce the need for close monitoring of universal coverage campaigns, where the true achieved net use might be significantly lower than the administrative measure of ownership would suggest.

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HIGH LEVEL OF SUBMICROSCOPIC INFECTIONS OF FOUR *PLASMODIUM* SPECIES DURING PRE-ELIMINATION PHASE IN NORTH SUMATERA, INDONESIA

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In the effort to eliminate malaria in Indonesia by 2030, the Indonesian Ministry of Health is implementing a four-stage elimination plan comprising different strategies across the archipelago. One strategy is to support all primary health centres with the capacity of malaria diagnostic testing. However, this strategy is limited by a surveillance system

which highly relies on passive clinical cases and diagnostic test using conventional microscopy examination. In the low malaria endemic areas which predominate in Indonesia, the true burden of malaria cases remains undiscovered, and the extent that asymptomatic and submicroscopic infections contribute to transmission is unknown. Therefore, this study aimed to determine the baseline epidemiological profile of *Plasmodium* species and to investigate the proportion of submicroscopic infections among all malaria cases in North Sumatera province, Indonesia. A cross-sectional survey was conducted in Batubara regency, Langkat regency and South Nias regency between January and June 2015. A total of 3635 participants were screened for *Plasmodium* infection by microscopy, rapid diagnostic tests, and molecular analysis using nested polymerase chain reaction. Primers targeting the *Plasmodium* small subunit ribosomal RNA were used to identify *Plasmodium* species, and an additional novel assay targeting the SICAvar gene was performed for *P. knowlesi* identification. All *Plasmodium* species except *Plasmodium ovale* spp. contributed to symptomatic and asymptomatic malaria cases in North Sumatera province. Overall positivity rate by nested PCR was 34.1% (1049/3080) with 10.8%, 17.1%, 6.5% and 13.3% positive for each *P. falciparum*, *P. vivax*, *P. malariae* and *P. knowlesi* infection. We found 61.1% of all malaria infections were not detected by microscopy, with *P. knowlesi* the most common submicroscopic infection. Despite the little known of the role of submicroscopic infection in malaria transmission, these results suggest that submicroscopic infections should also be targeted in malaria control and elimination programmes as they comprise a significant proportion of malaria infections.

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AN UPDATE ON EVIDENCE OF STRATEGIES TO PREVENT MALARIA IN PREGNANCY

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In sub-Saharan Africa, approximately 45% of the 32 million pregnancies occurring annually are estimated to be exposed to malaria infection, leading to an estimated 900,000 low birthweight deliveries. WHO recommends use of insecticide treated nets and intermittent preventive therapy with sulphadoxine-pyrimethamine (IPTp-SP) for prevention, though the latter is threatened by high levels of parasite resistance. Despite a decade's worth of intensive multicentre trials, the search for safe, effective, and well-tolerated alternative drugs to replace SP for IPTp has proven elusive. These have shown that neither amodiaquine, alone or combined with SP, mefloquine, or the fixed- dose combination of chloroquine-azithromycin are tolerated well enough to replace SP for IPTp in Africa. More recently, trials from Ghana, Malawi and Kenya, looked at intermittent screening and treatment in pregnancy (ISTp) as an alternative strategy to IPTp, consisting of intermittent rapid diagnostic testing (RDT) for malaria and treatment of RDT-positive cases with dihydroartemisinin-piperaquine (DP). The results from these trials were disappointing, showing either lack of cost-effectiveness in west Africa or up to 20% higher incidence of malaria during pregnancy in east/southern Africa, rendering this test-and-treat strategy as an unsuitable alternative to IPTp-SP in high SP resistance areas. However, two recent exploratory trials have shown promise for DP as IPTp in areas of high malaria transmission and SP resistance for reducing malaria infection (Incidence Rate Ratio [IRR]=0.32) and clinical malaria (IRR=0.16). The risk of fetal loss and early neonatal death was halved (61% to 47% lower) in the IPTp-DP arm, but the lower impact on fetal growth and preterm birth led to a more restricted overall pooled impact on "adverse pregnancy outcome" of 17% (95% CI 10-37%) using fixed effects meta-analysis. We will present results of a systematic review of evidence for prevention of malaria in pregnancy both in HIV-negative and HIV-positive women in sub-Saharan Africa, with a focus on the recent data and remaining gaps in knowledge.

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THE EPIDEMIOLOGY OF GENDER DIFFERENCES IN MALARIA UNRELATED TO PREGNANCY

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Although malaria is declining in India and Asia, it is still important to find out what factors are impacting prevalence and incidence, to assist in the development of effective strategies to reduce the burden. Malaria in pregnancy has been recognized as a clear risk factor for malaria, but it is not clear if there are additional differences by gender, and if these differences are related to exposure, or other factors. For decades it has been observed that gender differences in the prevalence of malaria exist in some areas in Asia. We examined gender differences in our studies in India, and combined our results with additional studies from the region identified in the literature using meta-analysis. Among clinic studies in five different sites in India, the pooled risk ratio (PRR) for men to be diagnosed with malaria compared to women was 1.83, 95% CI 1.48-2.27, I2 78% (n=12) for *Plasmodium falciparum*, and 1.92, 95% CI 1.72-2.13, I2 34% (n=12) for *P. vivax*. The difference was mainly among adults (any species: PRR 1.95, 95% CI 1.67-2.27, n=12), but not among children ≤15 years (any species: 1.05, 0.87-1.27, I2 42%, n=12). Results were similar for surveys in Asia, with a PRR of 1.55, 95% CI 1.16-2.06, I2 72% (n=7) for *P. falciparum*, and 1.25, 1.06-1.48, I2 0% (n=8) for *P. vivax*. Gender differences for malaria in the same direction were also noted in studies identified in the literature in Ethiopia and Uganda. We are currently in the process of adding information from the literature from other malarious areas in the world in a database to assist in an assessment of the most likely explanation for the gender differences seen in some parts of Asia.

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ASSOCIATIONS BETWEEN MEASURE OF MALARIA DURING PREGNANCY AND ADVERSE BIRTH OUTCOMES

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Malaria in pregnancy is associated with maternal morbidity, placental malaria, and adverse birth outcomes. However, data are limited on the relationships between longitudinal measure of malaria during pregnancy and outcomes measured at the time of delivery. Data came from a RCT of intermittent preventive therapy during pregnancy among 282 HIV uninfected women followed to delivery with histopathologic assessment for placental malaria. During pregnancy, malaria was measured using passive surveillance and asymptomatic parasitemia (AP) measured every month using a sensitive molecular assay (LAMP). Placental malaria was defined as either the presence of parasites in placental blood by LAMP or the presence of parasites or pigment detected by histopathology. Adverse birth outcomes included low birth weight (LBW, < 2500 gm) and preterm delivery (< 37 weeks). Associations were made between longitudinal measures of malaria during pregnancy and placental malaria and these same measures and adverse birth outcomes. Exposure to malaria during pregnancy was divided into 3 categories: 1) No malaria or AP (n=52, 18.4%), 2) 0-1 episodes of malaria and < 50% of samples positive for

AP (n=157, 55.7%), 3) 2-3 episodes or malaria or > 50% of samples positive for AP (n=73, 25.9%). The risk of placental malaria by LAMP was significantly higher among women in category 3 (25.0%, p=0.01) compared to categories 1 (1.9%) and 2 (3.2%). The risk of placental malaria by histopathology was significantly higher among women in categories 2 (29.9%, p=0.01) and 3 (74.0%, p<0.001) compared to category 1 (7.7%). Longitudinal measures of malaria during pregnancy were not associated with adverse birth outcomes. Placental malaria by LAMP was associated with significantly higher risk of LBW (45.8% vs. 10.2%, p<0.001) and preterm delivery (29.2% vs. 7.0%, p<0.001). Placental malaria by histopathology was not associated with adverse birth outcomes. Longitudinal measures of malaria during pregnancy were strongly associated with placental malaria but not adverse birth outcomes. Placental malaria by LAMP was associated with an increased risk of adverse birth outcomes.

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RECENT TRENDS IN MALARIA INCIDENCE AND SURVEILLANCE IN CAMBODIA

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Cambodia has the target of eliminating malaria by 2025. There are currently intensive efforts towards achieving this aim. However, there is great concern about the potential threat to elimination from increasing ACT treatment failure rates with artemisinin and ACT partner drug resistance. It is thus important to monitor trends in incidence rates of malaria in Cambodia and to understand their possible causes. Malaria surveillance data collected from multiple sources by the Cambodia national malaria control programme (CNM) from 2006 to 2016 were analysed. Overall there have been substantial decreases in malaria burden over this period, particularly for *P. falciparum*. The burden of *P. vivax* increased with roll-out of the village malaria worker (VMW) programme and wider availability of multi-species rapid diagnostic tests, peaking in 2011. In recent years, around half of malaria cases reported to CNM in Cambodia were diagnosed by VMWs. From 2013 to 2014, both falciparum and *vivax* malaria increased in incidence. This appears to have been at least partly due to a substantial increase in testing for malaria by village malaria workers (VMW) over the same period. In 2015, the number of people tested for malaria by VMWs fell and there was a decrease in the total number of cases of similar magnitude. In Western Cambodia, there was an increase in cases in 2015 due principally to a large rise in *P. falciparum* in only two operational districts with increased testing for malaria by VMWs, and a smaller increase in testing by health facilities. The data collection and analysis are ongoing and at the time of the meeting we aim to provide updated results and potential additional explanations for the observed trends. Studies are also underway to investigate the current rates of ACT treatment failure and antimalarial drug resistance and assess their potential impact on trends in malaria incidence. These preliminary results illustrate the important role of village malaria workers in diagnosing malaria in Cambodia and highlight the importance of maintaining a stable, high quality surveillance system to underpin efforts for malaria elimination.

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PREVALENCE OF MALARIA FROM BLOOD SMEARS EXAMINATION: A TWENTY YEAR RETROSPECTIVE STUDY FROM NATIONAL MALARIA REFERENCE LABORATORY, OUAGADOUGOU, BURKINA FASO

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Malaria is still a major public health problem in Burkina Faso. According to the Burkina Faso ministry of health statistic unit, malaria prevalence is increasing. In the WHO 2014 malaria report, the trend of malaria is decreasing in sub-Sahara. Therefore, the aim of this study was to determine real malaria morbidity based on the twenty year slide positive rate of malaria. A retrospective study was conducted at "Centre National de Recherche et de Formation sur le Paludisme" laboratory from 1990 to 2013. Twenty year malaria cases data had been collected from laboratory registration book. A total of 65464 patients were examined for suspected malaria; of these, 12922 (19.74%) had objective fever; 13159 (20.10%) study subjects were positive for malaria. A slide positive rate of *Plasmodium* within the last twenty years (1990-2013) decreased from 25.8% in 1990 to 9.3% in 2013 (p< 0,0001) with slight fluctuation during the study period. A rate of gametocytemia decrease from 3.52% in 1990 to 1.09% 1n 2013 (p< 0.0001). High slide positive rate of malaria occurred during high malaria transmission season 82.97%, followed by dry season 17.02% (p< 0.0001). The age groups of 5-14 years old were highly affected by malaria infection. During the study period, the trend was to the reduction. The predominant *Plasmodium* species detected was *P. falciparum* (97.83%) followed by *P. malariae* (1.61%).. In conclusion, slide positive rate of malaria was still high in study area. During the last twenty years, Plasmodic index & gametocytemia decreased based on the microscopy diagnosis. MoH should reinforce health system capacity to improve the data report.

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CHARACTERIZING THE IMPACT OF DYNAMIC VECTOR ABUNDANCE ON INDIVIDUAL MALARIA PREVALENCE IN A HIGH TRANSMISSION AREA OF NORTHERN ZAMBIA

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Vector control is a key strategy in reducing community malaria burden, however, little is known about the impact of vector population dynamics on individual malaria risk in multispecies systems. Malaria transmission in Nchelenge District, Zambia is complex due to the presence of two highly competent malaria vectors with distinct spatial and seasonal distributions, resulting in year-round hyperendemic transmission. From April 2012 to December 2015, the Southern Africa ICEMR enrolled 2,296 participants in active malaria surveillance and collected over 10,000 *Anopheles funestus* and 1,000 An. gambiae from indoor CDC light traps. Average mosquito counts per household were aggregated by species to each 1x1 km sampling grid by month of data collection. Multivariate spatial and temporal smoothing analyses will be conducted to further account for variation. A mean of 18.0 An. funestus and 1.7 An. gambiae were caught per household (range: 0-226 and 0-32). In preliminary regression analyses, mean An. funestus per household was significantly higher in the dry season, in inland areas, and in grids with higher use of insecticide treated nets (ITNs), and were lower in grids with greater coverage of indoor residual spraying (IRS). An. gambiae per household were correlated with climactic and environmental factors, including temperature, rainfall,

and altitude. Both species were highly predictive of individual malaria prevalence in multivariate logistic models. The odds of having a positive RDT increased 7.7% for each increase in 10 An. funestus (P=0.02) and 5.1% for each increase in 1 An. gambiae (P=0.04), controlling for clustering by household, age, ITN use, recent malaria treatment, history of IRS, education level, and distance to roads. Inclusion of environmental variables and distance to streams attenuated the association between malaria prevalence and An. gambiae and funestus, respectively, highlighting potential ecological drivers of mosquito and malaria dynamics. These findings illustrate the complexity of vector bionomics as a predictor of malaria risk and the need to target interventions to the unique ecology of each vector species.

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SPATIAL CHANGE IN THE RISKS OF *PLASMODIUM VIVAX* AND *P. FALCIPARUM* MALARIA IN CHINA, 2005-2014

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Despite the declining trend of malaria incidence over the last decades, *Plasmodium falciparum* malaria has increased in China both in terms of the number of case and geographical coverage. To help with the disease elimination, this study examined a spatial change in the risk of both *P. vivax* and *P. falciparum* malaria across China during 2005-2014. We applied logistic regression model to understand change in the risk of *P. vivax* and *P. falciparum* in each county across study period, and linear regression model to examine annual change in longitude and latitude of affected counties. The risk of *P. falciparum* malaria significantly increased with latitude and longitude, indicating that incidence rate of *P. falciparum* malaria increased in the northern and eastern, or decreased in south and western China. Similarly, latitude and longitude of counties with *P. falciparum* significantly associated with year. However, longitude of *P. vivax* affected counties significantly decreased, showing an annual declining number of *P. vivax* affected counties in eastern or increased in western China. The risk of *P. vivax* malaria had decreased whereas, the risk *P. falciparum* malaria had increased in the northern and eastern China. For successful elimination of malaria, underlying causes of the increased *P. falciparum* malaria risk needs further investigation.

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EVALUATING A REACTIVE TEST-AND-TREAT PROGRAM FOR SUB-PATENT MALARIA IN MACHA, ZAMBIA: OPTIMAL STRATEGIES TO ACHIEVE ELIMINATION

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In Choma District, Southern Province, Zambia, malaria prevalence by rapid diagnostic test (RDT) declined from 8% in 2008 to 1% in 2013. As part of an effort to achieve elimination, the Zambian government implemented a reactive test-and-treat (RTAT) program in parts of Southern Province in 2013. Individuals with confirmed malaria by health workers are followed-up within two weeks of diagnosis. All individuals living in households within 140 meters of the index case are tested with an RDT and treated if positive. This study aimed to optimize the RTAT strategy by characterizing infected individuals missed by both the RDT and the current screening radius. Health workers notified the study team of individuals with RDT confirmed malaria. For each study participant, a questionnaire was administered and a blood sample collected. To evaluate the optimal RTAT radius and assess the frequency of sub-patent, RDT negative infections, the radius was expanded to 250 meters and quantitative polymerase chain reaction (qPCR) testing was introduced. Spatial-temporal cluster detection was conducted to identify clusters of index households. From January 2015 to January 2016, 101 index cases were identified at health centers and health posts and followed up by the health workers with the study team. A total of 2504 individuals residing in 394 households were screened. Parasite prevalence was 2.5% by qPCR (53 positives of 2108) and 1.2% by RDT (26 positives of 2225). Of the qPCR positive cases, 66% of 53 individuals tested negative by RDT. 24 households had at least one qPCR+/RDT- individual. Nearly half of those infected resided within the index case household. The cluster detection revealed no clustering of index case households. The low number of secondary cases indicates low efficiency of RTAT beyond the index case household and the sensitivity of the RDT was too low to be an effective screening tool. Focal drug administration in which all individuals within index case households are treated may be a more efficient approach to achieving malaria elimination in southern Zambia.

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PREVALENCE OF ASYMPTOMATIC MALARIA INFECTIONS AND ASSOCIATED RISK FACTORS IN A HIGH TRANSMISSION REGION IN WESTERN KENYA

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Asymptomatic infections pose a major threat to malaria control programs since they act as silent reservoirs for the malaria parasites. Targeting the asymptomatic reservoir is key for the success of elimination efforts. We sought to determine the prevalence of asymptomatic malaria infections, whether they show heterogeneity and their determinants in a high transmission geographically homogenous region. This was part of a larger prospective cohort study that we set up in Bungoma East sub-County. We conducted quarterly parasitological surveys for a cohort of 400 participants from randomly selected households located in known fever 'hotspots' and "cold spots". Follow-up continued for a period of one year. Generalized estimating equations were used to model risk factors associated with asymptomatic parasitemia. A total of 321 malaria infections were detected during the five cross-sectional surveys over the course of one year. Almost half (46.3%) of these were asymptomatic. Overall, most of the asymptomatic cases (67%) were in households within known 'fever hotspots'. The proportion of infections that were asymptomatic in the coldspots were 73.1%, 31.8%, 13.3%, 55.6% and 48.2% during the first, second, third, fourth and fifth visits respectively. In the known fever hotspots, the proportion of infections without symptoms was 47.7%, 48.5%, 35%, 41.3% and 47.5% during the first, second, third, fourth and fifth visits respectively. Factors associated with asymptomatic malaria include; the village one lives: people living in Maruti village were twice likely to be asymptomatic (A.O.R: 2.141, C.I: 0.03 - 1.488), age: children aged between 6 to 15 years were more than twice likely to be asymptomatic (A.O.R: 2.67, C.I. 0.434 - 1.533) and the season: infections during the dry season (January) were less likely to be asymptomatic (A.O.R: 0.26, C.I: -2.289 - 0.400). The prevalence of asymptomatic infections in this region is very high. There is a need for active surveillance to detect the asymptomatic cases as well as treat them in-order to reduce the reservoir. Targeting interventions to the asymptomatic individuals will further reduce the transmission.

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GEOGRAPHIC DISTRIBUTION AND SPATIAL CLUSTERING OF SUBMICROSCOPIC MALARIA IN KAYIN STATE, MYANMAR

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In the Greater Mekong Subregion (GMS) malaria is spatially heterogeneous and seasonal. Since infections are relatively rare it is thought that few people should have asymptomatic and submicroscopic infections. However, recent work has revealed communities with high prevalences (>40%) of asymptomatic and submicroscopic malaria in several areas of the GMS. These preliminary studies focused on small numbers of villages and the distribution of such populations across landscapes in the GMS isn't known. The goal of this work was to assess and analyze the frequency and geographic distribution of submicroscopic malaria across four townships of Kayin State, Myanmar as part of a *Plasmodium falciparum* malaria elimination project. We used field-based geographic surveys to map a target area consisting of over 1,200 villages (approximately 16,500 km2). Villages were then randomly selected for blood surveys. Survey teams visited selected villages, randomly selected villagers for participation in the survey and took 2cc of whole, venous blood. Blood samples were analyzed using a highly sensitive quantitative PCR approach. Villages with an overall *Plasmodium* prevalence higher than 40%, of which 20% or more was falciparum malaria, were considered "hotspot" villages (43 villages out of 204 surveyed have been thus categorized so far). Clustering also occurs at scales larger than the community. Villages with high prevalences of either falciparum or *vivax* malaria occur near other villages with high prevalences. Most "hotspot" villages occurred within 5km of at least one other "hotspot" village. Finally, over 85% (37/43) of all "hotspot" villages occurred within a single subregion (less than 3,500 km2) of the overall target area. Our results indicate that submicroscopic malaria clusters at multiple scales. Populations with high prevalences of submicroscopic malaria may act as important reservoirs of the disease and could frustrate elimination efforts. These results have implications for planning and implementing interventions such as reactive screening and treatment or targeted mass drug administration.

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MALARIA PREVALENCE IN THE URBAN AREAS OF MANGALURU IN SOUTH INDIA

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Mangaluru city, located on the Southwestern coastal area of Karnataka State in India, is the government headquarter of Dakshina Kannada district. In late 1940s, malaria was common in the rural areas of the district with parasitemia rate of 5-12% and splenomegaly case rate of 17-46%, whereas incidences were low at urban areas of Mangaluru city. The malaria control program initiated in the district in 1948 succeeded in reducing the parasitemia and splenomegaly rates to 0.5% and 9.4% respectively by 1951. However, resurgence of transmission resulted in 440 deaths between 1956 and 1959, and with renewed control efforts, there were no deaths by 1961. From 1969 malaria started resurging again, reaching a peak of 5225 cases in 1975, and with the modified plan of National Malaria Eradication Program, it declined to 86 cases in 1988. Again, the numbers of malaria cases in the district increased to 340, 4027, 6692, 21159, and 11413, respectively, in 1991, 1993, 1995, 2005 and 2015; there were 26 deaths in 1995. While 95 (83%) of 114 cases reported in 1990 occurred in rural areas of the district, during 1993 to 2015, 86% cases have occurred in the urban areas of Mangaluru city and 14% in the rural areas. The average annual parasite incidence (API) has been 16 for Mangaluru city and 0.7 for rural areas of the district. The surges in malaria cases in Mangaluru city during 1995-97 and 2004-06 were accompanied by increased incidence in the adjoining rural areas. Industrial constructions, rapid expansion of urbanization and housing constructions, and migration of workers from malaria endemic areas are the likely causes for the resurgence of malaria in Mangaluru city, while the spread to rural areas of the district has been likely occurring through the people commuting from rural areas for work in Mangaluru city. The persistence of markedly higher API in the city compared to the rural areas suggests that Mangaluru city is a substantial malariagenic locality in the district. The increasing contribution of Mangaluru city to the total cases of malaria in Karnataka state, from 1.2% in 1994 to 67% in 2015, suggests the need for more effective control measures in Mangaluru city.

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MALARIA SURVEILLANCE DURING THE TRANSIT FROM CONTROL TO PRE-ELIMINATION

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The 2012 WHO manual of surveillance for malaria control proposed the monitoring of suspected cases, however the same manual fostered the use of confirmed malaria cases. On other hand to ensure that lab results do not influence the condition of "suspected" in the registers, has been proven to be a challenge. Following WHO guidance, Bioko Island Malaria Control Project (BIMCP) has been reporting the number of suspected cases, composed by adding a) the number of outpatients lab tested for malaria plus b) the number of presumed malaria cases (clinical diagnosis or diagnosed without lab confirmation). After 11 years of BIMCP's activity, Bioko is in transit from control phase to the pre-elimination, and a malaria surveillance system able to provide support for both phases is required. We have assessed the trends and composition of the group "suspected cases" to assess its contribution to the malaria surveillance. Between 2013 and 2016 the reduction in the average monthly number of suspected cases was quite slower (30%) compared with the decrease in its sub-group of confirmed malaria cases (75%), or the sub-groups with diagnostic behaviors potentially deviant of WHO guidelines (1. cases diagnosed as malaria without lab test, 2. cases diagnosed as malaria with negative test results, and 3. cases with a diagnosis other than malaria with a malaria test positive) which shown a decrease in the range of 68-71%. Conversely, among the suspected cases the average monthly number of cases with a malaria test negative and reported with a diagnosis other than malaria experienced an increase of 219%. We conclude that a) the number of suspected cases is not as sensitive as of confirmed cases to monitor the burden of disease, b) the training, supervision and availability of supplies for lab testing has allowed the reduction of undesirable diagnostic behavior, and c) clinicians may have in fact adopted more inclusive criteria to classify outpatients as malaria suspected cases along with the reduction of parasitemia in Bioko. NHIS data from at least the last four years will be analyzed to identify changes in the definition of suspected cases informally adopted by clinicians.

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THE RELATIONSHIP BETWEEN ANEMIA AND MALARIA INFECTION AMONG CHILDREN UNDER FIVE YEARS IN MALAWI

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Anemia is a public health concern in resource limited settings. Although the causes are multi-factorial, a major contributor is malaria. The World Health Organization has proposed using anemia prevalence as a surrogate measure of the burden of malaria infection. Despite the fact that several studies have demonstrated a correlation between the prevalence of anemia and the prevalence of malaria infection, none have explored the relationship between anemia and malaria prevalence over time or whether widespread rollout of malaria interventions is associated with a change in the prevalence of anemia. Six cross-sectional studies were conducted in southern Malawi during the rainy and dry seasons from 2012 to 2014. A mass distribution of insecticide-treated nets (ITNs) occurred after the first survey. Children 6 to 59 months of age for whom hemoglobin data were available were included in this analysis. Blood samples were collected by fingerprick, and hemoglobin was measured using Hemocue® photometer. Malaria infection was detected by qPCR. Zero-inflated Poisson mixed-effect regression models were used to assess the relationship between anemia prevalence and malaria prevalence or ITN use accounting for clustering at the neighborhood level. Hemoglobin data and PCR results were collected on approximately 3,100 children under 5 years of age. Malaria prevalence followed a seasonal trend with the highest prevalence in the rainy seasons. In contrast, anemia prevalence and ITN use did not show consistent patterns. Additional analyses are planned for confirmation. From our initial analysis, we found no evidence of association between changes in anemia prevalence and either changes in malaria infection prevalence or ITN use in children. Anemia may not be a useful surrogate for changes in malaria prevalence.

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ASSOCIATION BETWEEN CARRIAGE OF ASYMPTOMATIC INFECTIONS AND TIME TO CLINICAL MALARIA IN MALAWI: DATA FROM A LONGITUDINAL COHORT STUDY

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It is unknown whether asymptomatic *Plasmodium falciparum* infections lead to clinical malaria. However, there is some evidence that asymptomatic infections may protect against development of clinical disease. This study uses longitudinal data to examine the effect of asymptomatic infections on time to clinical malaria in a high-transmission region of Malawi. Individuals attending a health center with acute uncomplicated malaria were treated with artemether/lumefantrine (AL) and followed for up to 470 days. Blood samples were collected monthly for qPCR detection of infection. Passive surveillance for clinical malaria was conducted and, if RDT positive, participants were treated with AL. Mixed effect Cox models with time-varying exposure (asymptomatic infection) and random effects for repeated measures were used to estimate the effect of carriage of asymptomatic infection on time to clinical malaria. Models were adjusted for age, season, and ITN use. Follow up time for the current analysis began 30 days after each treatment with AL. There were 62 individuals with 192 periods of observation after a treatment, 137 ending in clinical malaria episodes and 55 censored observations. Median follow up time for all observations was 95 days (IQR = 39 - 202); 122 days (IQR = 51-265) for observations with asymptomatic infections and 59 days (IQR = 26 - 111) for observations without asymptomatic infection. After adjustment, individuals with asymptomatic infections had an increased time to clinical malaria compared to individuals without asymptomatic infection (Hazard Ratio = 0.67, $p < 0.05$). Asymptomatic infections are associated with decreased risk of clinical disease compared to those without asymptomatic infection. This could be attributed to a protective effect of asymptomatic infection against symptomatic malaria. Alternatively, the ability to maintain an infection without symptoms may be an indicator of host immunity. Understanding the mechanism that underlies the relationship between asymptomatic infection and clinical disease is essential to developing interventions and may provide insight into the basis of acquired immunity.

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COST ANALYSIS OF AN LLIN KEEP-UP CHANNEL IN TANZANIA: THE SCHOOL NET PROGRAM

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Although school distribution has been named by World Health Organization as a channel for maintaining universal coverage of long lasting insecticide treated bed nets (LLIN), little is known about the cost or cost-effectiveness of this approach. In 2011-2012, Tanzania attained universal coverage, reaching over 90% ownership of at least one LLIN. Since then, in the southern zone LLINs have been distributed to school children in primary school classes (1, 3, 5 and 7) and secondary school classes (2 and 4) once a year as a keep-up strategy. A cost analysis of the third round of the School Net Program (SNP3) was implemented in 2015. During SNP3, the government distributed 494,407 LLINs to 1,919 schools in 19 districts. The study utilized the provider perspective and estimated both economic and financial costs. Costs were collected retrospectively from financial and operational records and through stakeholder interviews at the national and regional level. A survey instrument was utilized to collect resource use and expenditure information at the district and school level. Fifteen out of the 19 districts were sampled along with two schools from each of those districts. Average costs for each activity were calculated from the sampled districts and schools and applied to those not included in the sample. Overall, SNP3 was able to deliver LLINs at an economic cost of 7.70 USD per net distributed during 2015. This translates into an estimated economic cost per person year of protection of 1.28 USD. Of the total economic cost, approximately 4.33 USD (56%) was for distribution while the remainder of costs related to the cost of the net itself. These costs, as well as the cost per treated net year and cost per person year of protection appear comparable to other LLIN continuous distribution systems.

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DO MALARIA HOTSPOTS REALLY FUEL TRANSMISSION?

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Spatial heterogeneity in malaria transmission has been recognized for some time and the idea of using these 'hotspots' of intense transmission for targeting control and elimination interventions have received considerable attention. However, despite the biological plausibility of the hotspot idea, both in terms of these areas seeding transmission and that targeting these areas with interventions can have a disproportional impact on transmission, the evidence to support this concept has been mixed. Evidence suggests that some hotspots are consistent between years while others are not which has important implications for how to inform control practices. Similarly, malaria heterogeneity exists on all scales and transmission intensities however; identifying when and whether identifying hotspots becomes operationally feasible is unknown. To study the spatial and temporal dynamics of malaria transmission, data from a longitudinal cohort in The Gambia was analyzed. All consenting individuals residing in twelve villages across the country were sampled monthly from June (dry season) to December (wet season) 2013. A study nurse stationed within each village recorded all malaria episodes between visits. *Plasmodium falciparum* infections were determined by polymerase chain reaction. Spatial and spatio-temporal analysis was conducted using the PrevMap package in R. Results indicate that malaria is focal around high burden households suggesting that such households seed transmission. However, this pattern was only observed in low transmission villages. As transmission intensity increases, households with a consistently high burden of malaria exist, but the high burden households change over time and infections are present in the majority of houses making a targeting strategy less appropriate. This study provides the first detailed assessment of the transmission patterns at the village level and has important implications for understanding the applicability of spatially targeted control approaches in this setting.

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HEALTH WORKER ADHERENCE TO MALARIA CASE MANAGEMENT GUIDELINES AT PUBLICLY FUNDED OUTPATIENT HEALTH FACILITIES — SOUTHERN MALAWI, 2015

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Six million episodes of malaria occur in Malawi annually. Gaps in quality malaria treatment persist. We conducted a cross-sectional outpatient health facility (HF) survey in southern Malawi in January–February 2015 to identify opportunities for promoting adherence to the 2013 national malaria case management (CM) guidelines. Using the 2013 CM guidelines, surveyors classified patients as suspect uncomplicated or suspect severe malaria. Main outcomes were appropriate testing (suspect uncomplicated malaria only) and treatment (confirmed or suspect uncomplicated and suspect severe malaria) based on the CM guidelines. Weighted descriptive and logistic regression analyses of patient, health worker (HW) and HF characteristics were performed. We evaluated 105 HFs and interviewed 150 HWs and 2342 patients. Of 1427 suspect uncomplicated malaria patients at HFs with malaria testing, 1072 (75.7%) were tested, and 547 (53.2%) tested positive. In total, 511 (92.7%) confirmed and 98 (60.3%) suspect malaria patients (at HFs without testing) were appropriately treated. Only 8 (5.7%) suspect severe malaria patients received appropriate pre-referral treatment. Patients were more likely to get tested for malaria if they reported fever (odds ratio [OR] = 2.6; 95% confidence interval [CI]: 1.7–4.0), headache (OR = 1.5; 95% CI: 1.1–2.1) or vomiting (OR = 2.0; 95% CI: 1.0–4.0) to HWs and less likely to be tested if they reported a skin problem (OR = 0.4; 95% CI: 0.2–0.6). For patients with suspect malaria, appropriate treatment was more likely with elevated temperature (OR = 1.5 per 1°C increase; 95% CI: 1.2–1.9), patient reported fever (OR = 7.2; 95% CI: 2.6–20.2), being seen by HWs with a copy of the 2013 malaria CM guidelines (OR = 11.8; 95% CI: 3.9–34.8) or HWs with additional supervision visits in the last 6 months (OR = 1.3 per additional visit; 95% CI: 1.1–1.5), but less likely for those attending rural hospitals versus health centers (OR = 0.2; 95% CI: 0.1–0.3). HW solicitation of patient symptoms may improve malaria testing practices. Appropriate treatment of malaria may be improved by extra supervision visits to HWs, plus better access to tests and guidelines.

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THE CHANGING BURDEN OF MALARIA IN PREGNANCY AND CURRENT EFFECTIVENESS OF INTERVENTIONS

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The risk of malaria in pregnancy (MiP) has fallen considerably in the 21st century as interventions have successfully reduced prevalence within the general population. Combining an existing mathematical model of MiP with estimates of endemicity between 2000 and 2015, we estimate that the risk of MiP has fallen on average by 37% across all areas of sub-Saharan Africa at risk. The per-pregnancy risk of MiP-attributable low birthweight (LBW) faced by women not covered by interventions such as Intermittent Preventative Treatment (IPTp) with sulfadoxine-pyrimethamine (SP) has fallen by 32%. However, the risk of MiP remains substantial: in the absence of pregnancy-specific intervention we estimate 8.5 million women would have experienced MiP leading to 660,000 LBW deliveries in 2015. We combined these estimates of MiP risk with current intervention coverage from population based surveys. Across 26 countries median use of LLINs in multigravidae (1 previous child or more) was 40%, but in primigravidae was substantially lower (median 25%). Only 21.1% of pregnant women at risk are receiving any IPTp, lagging well behind antenatal clinic (ANC) attendance (64.1% of these women attended ANC at least three times). We then combined our maps of the MiP burden with maps of SP resistance mutations. IPTp efficacy in different areas was estimated based on IPTp studies in areas with different mutation frequencies. Based upon 2010 levels of resistance, 9 million women reside where IPTp-SP is still likely to be highly effective and attended ANC at least three times during pregnancy, but did not receive any IPTp. This represents 200,000 LBWs which could be averted highly cost-effectively. We found 24% (6.2 million) deliveries occurred in settings where the quintuple SP resistance mutation had saturated by 2010, with 2 million occurring in settings with appreciable levels of the sextuple haplotype. Increasing LLIN use, particularly in primigravidae, and providing IPTp to women already attending ANC should be key priorities. Increased monitoring of the effects of SP resistance, as well as research into suitable alternatives to current IPTp regimens is also crucial.

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PREVALENCE OF ASYMPTOMATIC MALARIA AND ANAEMIA AMONG SCHOOL AGE CHILDREN IN TWO ECOLOGICAL ZONES IN GHANA

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Asymptomatic Malaria in school children is a public health challenge because of its implication in anaemia and cognitive functioning of school children. We compared the prevalence of *Plasmodium* spp. and anemia among school children in the forest and the coastal zones of Ghana over a three year period. A cross sectional survey was conducted yearly for 3 years in randomly selected schools in a coastal savanna town (Cape Coast) and a town located in the Forest zone (Begoro). A total of 2751 children aged 6 to 14 years were screened between 2013 and 2015 during the rainy season (September to November). The pupils provided their clinical and medication history two weeks prior to the screening and their heights body weights and axillary temperatures were taken. Each pupil provided a finger-prick blood for the preparation of thick and thin blood films for detection of parasitaemia by microscopy. The overall prevalence of asymptomatic malaria ranged from 17.5% to 23.1% over the 3 years period. The prevalence was higher in Forest zone (Begoro) than in coastal savanna town (Cape Coast) consecutively over the 3 years however significant deference was only observed in 2014 with a prevalence of 24.2% and 11.3% respectively (P value < 0.0001). Highest prevalence of malaria was observed in children age between 9 and 11 years (36.5% in 2013, 43.1% in 2014 and 42.1% in 2015). *P. falciparum* is the predominant species in both sites (79%, 86.2% and 94.3% for 2013 to 2015 respectively). Other species identified in Begoro (forest zone) were *P. malariae* and in Cape Coast were *P. malariae* and *P. ovale*. The prevalence anaemia were 10.2%, 14.5% and 16.5% respectively from 2013 to 2015. Anaemia was associated with asymptomatic malaria (OR=0.42, p value <0.0001 in 2013, OR=0.62, p value = 0.049 for 2014 and OR=0.46, p value <0.0001 for 2015). We conclude that malaria parasite prevalence is relatively high among school children in the coastal and forest zones of Ghana. Anaemia in school children increased from 2013 to 2015 and is associated with asymptomatic malaria. Asymptomatic malaria in basic schools can be used to monitor the effectiveness of the malaria intervention.

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FAILURE OF AVAILABLE MALARIA CONTROL INTERVENTIONS IN DANGASSA, MALI: CONTINUOUSLY HIGH PREVALENCE OF *PLASMODIUM FALCIPARUM* INFECTION IN A COHORT OF 1,400 INDIVIDUALS FROM 2012 TO 2015

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During the past decade, major investments have been made in sub-Saharan Africa leading to improved case management, greater coverage and use of LLINs and scale up of Seasonal Malaria Chemoprevention (SMC). We have examined changes in the seasonal prevalence of *Plasmodium falciparum* infection over a 4 year period in the context of malaria control interventions such as universal coverage with LLINs, IPTp for pregnant women, free ACT treatment for malaria and have recently introduced SMC. Our hypothesis is that long-term carriage in asymptomatic subjects is essential to maintain transmission in such populations/conditions. From 2012 to 2015, seven (7) blood smear surveys were performed in both the dry and rainy seasons in a well characterized cohort of adults and children in Dangassa (n=1500). Clinical and parasitological measurements to detect both symptomatic and asymptomatic infections were performed during each survey. Asymptomatic infection was defined as a positive smear for *P. falciparum* asexual parasites with an axillary temperature ≤ 37.5o C and no history of fever in the previous 24 hours. Seven point-prevalence of asymptomatic carriage across age groups and among pregnant women was assessed. The overall prevalence of asymptomatic infection varied from 13.3% to 51.3% in June 2015 and October 2015. Symptomatic carriage was most frequent at the end of the rainy season with 12.2% in 2012 and 11.9% in 2014. High frequencies of asymptomatic infection were observed in the dry season for children 5 to 14 years of age: 57.2% in 2013 and 64.5% in 2014. Prevalence of asymptomatic infection was also higher in pregnant women during the low transmission varying from 24.3% in 2013 to 28.6% in 2014. In conclusion, these results suggest that in Dangassa, despite multiple interventions against malaria, the prevalence of asymptomatic *P. falciparum* infection remains high, emphasizing the need for additional control approaches targeting the dry season reservoir and persistent transmission in order to reduce the continuing burden of malaria.

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TREATMENT SEEKING BEHAVIOR, DIAGNOSIS AND TREATMENT PRACTICES IN TANZANIA: COMPARISON BETWEEN COMMUNITY SURVEYS CONDUCTED SOON AFTER THE IMPLEMENTATION OF THE AFFORDABLE MEDICINES FACILITY - MALARIA AND THE MRDTS ROLL OUT, AND THREE YEARS LATER

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Considerable efforts have been made to increase access to first-line antimalarials and mRDTs across Africa. In Tanzania mRDTs were rolled out in the public sector from 2009-12, while ACTs are subsidized in the public and private sectors. We present findings from community surveys conducted in 2012 and three years later to assess the degree to which these interventions have led to sustained changes in case management. Both surveys took place in the same regions of Tanzania (Mwanza, Mbeya and Mtwara) after the rainy season. In each region, community surveys were conducted in randomly selected wards of urban and rural districts. Health seeking behavior and drug use were inquired from individuals reporting fever in the previous two weeks. All participants were tested for malaria using mRDTs. Between 2012 and 2015, parasite prevalence increased from 16% (1428/8834) to 21% (497/2351) in Mwanza, 2% (125/5941) to 4% (82/1980) in Mbeya and 16% (837/5327) to 26% (554/2138) in Mtwara. A larger proportion of febrile cases who sought treatment were tested in 2015 (20% (210/1037)) than in 2012 (10% (169/1653)). This also translates into more antimalarials delivered. Results from both surveys show that in private as in public sectors, febrile cases were more likely to be treated than tested for malaria (14% (379/2690) tested and 35% (949/2690) received an antimalarial), especially in regions of high parasite prevalence. The majority of antimalarial therapies were obtained from private sector (25% (669/2690) and 10% (280/2690) in public sector), which is also the sector where the febrile cases were the least tested (10% (108/1096) of individuals seeking treatment in private sector tested against 55% (195/354) in public sector). Despite increasing between 2009 after roll-out of RDTs and 2012, testing still remains poorly used, especially in private sector. Increase of drug use consumption may

reflect better health seeking behavior but probably more a remaining overuse of antimalarials. These results highlight the importance of targeting the private sector for improving and encouraging the use of mRDTs in order to ensure rational and adequate use of antimalarials.

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ACCESSING *VIVAX* MALARIA WITH OVERLOOKED GENES: THE DIVERSITY OF VIR GENES IN *PLASMODIUM VIVAX* FROM NORTHERN REPUBLIC OF KOREA

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Plasmodium vivax is the most prevalent human malaria parasite causing most of the cases outside of sub-Saharan Africa. Since 1993, *P. vivax* has been consistently arising in the northern area of Gyung-gi and Gang-won, Republic of Korea (ROK). The vir proteins, variant surface antigens (VSAs) of *P. vivax*, are considered as the key proteins to escape a host immune system through their antigenic variation, to infect erythrocyte's surface, and partially to induce the adherence of *P. vivax*-infected reticulocyte to the endothelial cell receptor. In these regards, vir genes were proposed to be the candidates for a target of vaccine development, but variant nature of VSAs have been an obstacle to develop a potent vaccine. In this study, genetic diversity of four vir genes, vir 27, vir 21, vir 12, and vir 4, was evaluated using 85 venous blood samples collected from *vivax* malaria patients in 2011-2013, ROK. The number of SNPs was distributed from low as 5 (vir 4) to high as 143 (vir 21). The average number of haplotypes of all vir genes was 8.25, and average Hd was 0.727. Vir 12 (H= 9, Hd= 0.795) showed the most genetically diverse followed by vir 21 (H= 9, Hd= 0.752), vir 27 (H= 6, Hd= 0.774), and vir 4 (3, 0.530,). Tajima`s D values of vir 27 (1.08530, $P > 0.1$), vir 12 (3.22553, $P < 0.01$), and vir 21 (0.52098, $P > 0.1$) were all positive, meaning decreased size of these genes' population and process of balancing selection. Moreover, Tajima`s D value of vir 12 was statistically significant, indicating that vir 12 was under balancing selection and/or decreasing population size. This study was the first survey about the vir gene in ROK, which provides the information of vir gene in ROK on genetic level. Among the four vir genes, it seemed that the most divergent gene was vir 12 and the vir 4 gene the most conserved. However, the sample sequences used in this study have shown a clear difference with Sal 1 reference gene sequence, while vir 4 gene was very similar to Indian isolate.

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PACBIO TECHNOLOGIES FACILITATE GENERATION OF A HIGH-QUALITY *PLASMODIUM COATNEYI* GENOME SEQUENCE AND ASSEMBLY

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High-quality genome sequence assembly and annotation are imperative for system biology-based studies to understand the complexity of parasite-host interactions in malaria. After decades of research, the first *Plasmodium* whole genome sequence, *P. falciparum* was published in 2002 and both genome and other technologies have been advancing ever since, to the benefit of research aimed at understanding the biology of the parasite and the disease. *Plasmodium coatneyi* infection of rhesus macaques is an exceptional model to study malaria severity, given the biological and clinical features that are shared with *P. falciparum*. However, a complete genome sequence and annotation of *P. coatneyi* has not been available. As a solution, we developed a *P. coatneyi* assembly using PacBio (RS SMRT®) sequencing. This long-read technology (at least 10kb) has resulted in an assembly with high coverage and statistical confidence, with only one gap existing in the parasite's14 chromosomes. A technology comparison as well as an evaluation of the current *P. coatneyi* genome sequences (nuclear, mitochondrial, and apicoplast), gene annotation, and repetitive regions will be presented.

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GENETIC POPULATION STRUCTURE IN HOTSPOTS OF *PLASMODIUM VIVAX* INFECTIONS IN THE PERUVIAN AMAZON: CLOSING THE GAP BETWEEN GENETICS AND EPIDEMIOLOGY

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Molecular analysis and spatial statistics provide new insights toward understanding the complexity of malaria transmission in the Peruvian Amazon as well as to better design interventions to move toward elimination. In this study, we evaluated the genetics of parasite populations in hotspots of *Plasmodium vivax* detected by microscopy in a seasonal endemic community (Lupuna, Iquitos) of the Peruvian Amazon. A population-based surveillance was conducted from March 2013 - March 2014. Every three months, we georeferenced houses and took blood samples for light microscopy examination. Samples on filter paper were collected by finger-prick for RT-PCR diagnostic and microsatellite (MS) genotyping. Of 4,090 samples (902 individuals) collected, 210 were positive by microscopy and 154 were analyzed with 9 previously reported and 7 new MS. Although four main subpopulations were identified by Bayesian analysis (STRUCTURE v2.3.4), only subpopulation 4 was stable across all surveys. Statistically significant hotspots with Satscan v9.4.2 (applying a Bernoulli model) were identified in every survey except in December (low transmission). A logistic regression showed that infections inside hotspot (n=31.2%; RR=2.62; p=0.024) during the high transmission month of June were two times more likely to be from subpopulation 4 than those located outside hotspot (OR=2.73; 95%CI=1.02-7.29). Concurrent vector biology analysis determined that in June, indoor and outdoor human biting rates by *Anopheles darlingi* decreased dramatically, supporting the hypothesis of bottleneck and clonal expansion of subpopulation 4 despite introduction of many other parasite populations. Our study findings confirmed the endogenous parasite population source of local transmission and hotspots in riverine Amazonian communities.

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TEMPORAL DYNAMICS OF GENOME-WIDE TRANSCRIPTION IN MALARIAL CHILDREN IN BURKINA FASO

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Malaria is a complex infectious disease with many genetic and environmental determinants influencing host immune response to infection, the progression of the disease and its severity. To date, surveys of host transcriptional responses used largely on cross-sectional study designs. Here we investigate the temporal dynamics of peripheral blood

transcription response to *Plasmodium falciparum* infection using RNA sequencing of a pediatric cohort in Burkina Faso. Children from 2-10 years living in two malaria-endemic villages of Banfora health district in Burkina Faso were sampled between May and November 2015 before and during the course of infection. Hundred bp Paired-End RNASeq profiles were generated and sequenced on an Illumina HiSeq instrument. Supervised statistical analysis of host genome-wide gene expression profiles revealed the nature, magnitude and significance of transcriptional changes taking place during the course of malaria infection. Gene set and pathway enrichment analysis identified key signature pathways, molecular and biological processes of host immune system modulated in response to infection. Integrated genotype and gene expression analysis allowed quantification of the contribution of host genotype to response to infection. The study provides a high-resolution picture of temporal transcriptional changes in a highly malaria-endemic region.

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GENOMIC SIGNALS OF CHANGING MALARIA TRANSMISSION

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Levels of genetic variation in the malaria parasite *Plasmodium falciparum* are known to vary by transmission intensity. The chance of the exact same multi-locus pattern of variation occurring several times in the population tends to be very low when transmission is intense, due to the increased opportunity for sexual reproduction and recombination. In contrast, in regions approaching elimination the same haplotypes tend to be found repeatedly due to clonal propagation. The tipping point at which this change in the population occurs is a crucial consideration for surveillance programmes, where genetic technologies are likely to play an increasingly important role in future. Here we use a fully integrated model of malaria genetics and epidemiology to explore the bottlenecks in the *P. falciparum* lifecycle that are capable of generating the observed patterns. Our results are able to capture the major trends in genomic variation over a range of transmission intensities, while also providing insight into the relative strengths of the different processes that lead to a loss of variation in decreasing transmission. We apply this model to a range of elimination and pre-elimination scenarios to explore the ways in which malaria epidemiology can influence and shape parasite population genetics. These results add to an expanding body of evidence that highlight the importance of genetic data as part of future control and elimination schemes.

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DEVELOPMENT OF A MULTIPLEX QPCR ASSAY FOR THE QUANTITATION OF *PLASMODIUM FALCIPARUM* GAMETOCYTOGENESIS IN A COHORT OF ASYMPTOMATICALLY INFECTED ADULTS IN WESTERN KENYA

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Plasmodium falciparum gametocytogenesis and gametocyte carriage remain poorly understood in areas of endemic malaria transmission, in spite of the important correlation between malaria transmission and gametocyte carriage. A sensitive and efficient method for quantitation of gametocytes in field samples is needed to determine transmission potential and evaluate mechanisms leading to increased gametocyte production. A multiplex quantitative PCR (qPCR) assay is well suited for this purpose because it uses multiple different fluorescent probes to simultaneously quantify the expression of several markers, reducing the amount of time and sample needed as compared to running multiple individual assays, and it is capable of detecting even very low levels of gene expression. Using the TaqMan qPCR chemistry, we are developing and validating a multiplex assay for simultaneous quantification of the expression of *Pfs25*, *Pfs230p*, and *PfAP2-G*. *Pfs25* and *Pfs230p* are markers of female and male gametocytes, respectively, and are being quantified because the ratio of female to male gametocytes can have implications on transmission, and because together, they represent total gametocytemia. *PfAP2-G* is an important master regulator of gametocytogenesis, and its expression correlates strongly with gametocyte commitment in cultured parasites. Additionally, we are further validating the utility of the multiplex assay by quantifying the expression of our markers of interest in a large cohort of adults with asymptomatic *P. falciparum* infections in western Kenya. Multivariate analysis of this expression data could revel trends in gametocyte development in this population. In turn, a better understanding of gametocyte development and prevalence could inform vector control programs to focus resources on the areas with the highest transmission potential, and educate clinical decision making to benefit patients while also reducing malaria transmission.

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TURNING BACK THE CLOCK: A HISTORY OF APICOMPLEXAN SPECIES DIVERGENCE

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An accurate timescale of the evolutionary history of apicomplexan parasites is critical for understanding rates of speciation, interactions between parasites and their hosts, the acquisition of new virulence traits, and the emergence of new diseases. Despite the tremendous public heath, socio-economic and agricultural impact of diseases caused by apicomplexan parasites such as *Plasmodium*, *Cryptosporidium* and *Theileria*, key phylogenetic aspects remain controversial or unknown, and an extensive estimate of the age of speciation of many lineages has never been attempted. Some studies have calibrated divergence times with substitution rate estimates for rRNA or organellar genes, which are not adequate for all levels of divergence, and may not be representative of the entire genome. Recently, we developed and validated a novel statistical approach to estimate the age of speciation events, using genome-wide protein sequence divergence. We used this approach to date the relative age of evolutionary lineages of several mammal-infecting *Plasmodium* species. Here, we re-apply this method to updated and expanded *Plasmodium* genomic data, including avian parasites, and further widen the taxonomic breadth of the study to include other genera, such as *Cryptosporidium, Theileria* and *Eimeria*. We find that protein divergence within genera, but not between genera, can be aptly described by a molecular clock model, and provide a comprehensive timeframe for the evolution apicomplexan parasites that yield novel insights into the co-evolution of these species and their hosts.

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DETERMINATION OF THE *PLASMODIUM VIVAX* RECURRENCE PATTERN IN INDIVIDUALS OF THE COMMUNITIES OF CAHUIDE AND LUPUNA OF THE PERUVIAN AMAZON

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Plasmodium vivax is a major cause of malaria in Perú, and most cases are concentrated in the region of Loreto. The presence of latent hypnozoites makes it difficult to classify recurrent *P. vivax* infections as relapses, recrudescences or re-infections. The characteristic of the different

recurrences has not been defined adequately in this region despite obvious relevance to malaria control and elimination. This study determined the *P. vivax* recurrence pattern in individuals of the communities of Cahuide (CAH) and Lupuna (LUP) in the region of Loreto. A total of 50 individuals of both communities with *P. vivax* recurrence that were followed up monthly from August 2012 to March 2014 were screened for parasitemia determination by qPCR 18S rRNA and subsequently all *P. vivax* infections were genotyped using 17 microsatellites. STRUCTURE analysis were performed and the recurrences pattern were determined. After the analysis only 44 individuals where evaluated for recurrence pattern, 24 from CAH and 20 from LUP; of 24 individuals of CAH 6 individuals had one homologous recurrence, 15 had one heterologous recurrence, 1 had two homologous recurrences, 1 had two heterologous recurrences and 1 had three heterologous recurrences. In LUP of 20 individuals 6 had one homologous recurrence, 13 had one heterologous recurrence and 1 had one homologous and one heterologous recurrence. In both communities, the presence of individuals with recurrent *P. vivax* infections were observed, the majority of these cases were heterologous recurrences and most of them were detected in the seasons of intense malaria transmission of this region. Finally the parasitemia in recurrences were lower than first episode despite the greater amount of heterologous recurrences which could indicate that although they can be reinfections these would generate an immune response that controls the parasitemia.

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A PARASITE GENETICS APPROACH TO EVALUATE MALARIA TRANSMISSION DYNAMICS IN ZAMBIA

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Malaria endemicity is highly heterogeneous in Zambia with *Plasmodium falciparum* prevalence by rapid diagnostic test ranging from 50% in Nchelenge District, located in northern Zambia, to 1% in Choma District in the south. Committed to malaria elimination by the year 2020, Zambia is faced with developing control strategies which will be effective in diverse epidemiological settings. Moving forward, it will be critical to understand the mechanisms by which transmission is sustained in these various settings. In particular, we are interested in evaluating the extent of cross-border transmission between Nchelenge District and the Democratic Republic of Congo (DRC). Parasite genetics offers a means through which we can assess this threat to local control efforts and better understand transmission dynamics in Zambia. We performed amplicon deep sequencing at the var2CSA locus of *P. falciparum* samples collected from Nchelenge District, Zambia and Haut-Katanga District, DRC, on the opposite side of Lake Mweru. Deep sequencing allows us to enumerate the haplotypes present in a given population. Subsequent comparisons of genetic relatedness and diversity between samples, including Principal Component Analysis will enable us to evaluate genetic diversity with the aim of determining the extent to which cross-border transmission occurs between Nchelenge District and the DRC.

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ORIGIN AND SPREAD OF *PLASMODIUM VIVAX* AND *P. FALCIPARUM* IN THE AMERICAS

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Plasmodium falciparum is thought to have been introduced in the continent from Africa, over the past 400 years, as a result of to the transatlantic slave trade, although evidence to support this hypothesis is lacking. The date(s) and routes of spread of *P. vivax* to the Americas is unknown. Here we examine patterns of genetic divergence between malaria parasite populations from the Americas and other continents with the purpose of inferring their origins and time of divergence from putative founding populations. We sequenced the mitochondrial genome of 114 isolates of *P. vivax* (including 9 isolates from howler monkeys from southeast Brazil that have been identified as *P. simium*) and 223 isolates of *P. falciparum* from different continents and compared these data with publicly available sequence data. Most *P. vivax* parasites from the Americas clustered together, in phylogenetic analysis, with samples from Africa and South Asia, consistent with a recent spread of this species throughout these regions. However, one highly differentiated cluster (including mostly samples from Eastern Brazilian Amazon and Venezuela) was observed, which may suggest an independent introduction (but geographically restricted) of this parasite in the Americas. *Plasmodium* I samples clustered together, being clearly differentiated from the rest of American *P. vivax* samples. There greatest *P. falciparum* diversity was found in South Asia and Africa, while South American parasites were the least diverse. Interestingly, nearly all *P. falciparum* samples from the Americas clustered together and were clearly differentiated from those from other continents, consistent with a major population bottleneck during the (recent) introduction of this parasite into the Americas.

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EVOLUTION OF THE TRANSMISSION-BLOCKING VACCINE CANDIDATES PVS28 AND PVS25 IN *PLASMODIUM VIVAX*: GEOGRAPHIC DIFFERENTIATION AND EVIDENCE OF POSITIVE SELECTION

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Transmission-blocking (TB) vaccines are considered an important tool for malaria control and elimination. Among all the antigens characterized as TB vaccines against *Plasmodium vivax*, the ookinete surface proteins Pvs28 and Pvs25 are leading candidates. These proteins likely originated by a gene duplication event that took place before the radiation of the known *Plasmodium* species to primates. We report an evolutionary genetic

analysis of a worldwide sample of pvs28 and pvs25 alleles. Our results show that both genes display low levels of genetic polymorphism when compared to the merozoite surface antigens AMA-1 and MSP-1; however, both ookinete antigens can be as polymorphic as other merozoite antigens such as MSP-8 and MSP-10. We found that parasite populations in Asia and the Americas are geographically differentiated with comparable levels of genetic diversity and specific amino acid replacements found only in the Americas. Furthermore, the observed variation was mainly accumulated in the EGF2- and EGF3-like domains for *P. vivax* in both proteins. This pattern was shared by other closely related non-human primate parasites such as *Plasmodium* cynomolgi, suggesting that it could be functionally important. In addition, examination with a suite of evolutionary genetic analyses indicated that the observed patterns are consistent with positive natural selection acting on pvs28 and pvs25 polymorphisms. The geographic pattern of genetic differentiation and the evidence for positive selection strongly suggest that the functional consequences of the observed polymorphism should be evaluated during development of TBVs that include Pvs25 and Pvs28.

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ASSOCIATION BETWEEN THE ALPHA THALASSEMIA TRAIT AND *PLASMODIUM FALCIPARUM* GAMETOCYTEMIA IN A MALARIA ENDEMIC AREA IN GHANA

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The gametocyte stage of *Plasmodium falciparum* represents an important stage in human/vector malaria transmission and a highly relevant target for disrupting transmission. The alpha thalassemia trait has been associated with protection against severe malaria, however its influence of parasitemia carriage remains unclear. This study aimed to determine the prevalence of *P. falciparum* asexual stage parasitemia and gametocytaemia in association with alpha thalassaemia. The study involved three cohorts of children, non-pregnant male and female adults, and pregnant women from Asutsuare, a malaria endemic community in Ghana. The prevalence of sub-microscopic gametocytaemia was detected by Pfs25 real time PCR. The prevalence of α-thalassaemia was determined by genotyping the African α -globin deletion, α 3.7, by PCR. Children with wild type or heterozygous α-thalassemia were two times less likely to harbour parasites, compared with those having the mutant allele (OR; 0.52; 95% CI, 0.28-0.97; P=0.037), however, this association was not present in adults and pregnant women. Sub-microscopic gametocytaemia prevalence was 39.5% in children, 29.7% in pregnant women and 8.7% in adults. There was no association between any α-thalassemia allele and gametocyte carriage in any of the cohorts. In conclusion, children and pregnant women had higher asymptomatic parasitemia and may potentially serve as reservoirs of transmission. Wild type and heterozygous α-thalassemia alleles were protective against asymptomatic parasitemia in children but not in adults and pregnant women. This study found no association of alpha-thalassaemia with increased risk of gametocyte carriage, perhaps due to the limited data on the gametocyte prevalence in the study community.

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PLASMODIUM VIVAX ISOPRENOIDS BIOSYNTHESIS PATHWAY ENZYMES AS PROBABLE DRUG TARGETS

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Malaria remains one of the world's major infectious disease imposing significant economic burden on various developing countries. Among the major *Plasmodium* species causing human malaria, *Plasmodium vivax* is now known to cause severity similar to that caused by *P. falciparum*, which has attracted a lot of attention towards the parasite. There are reports on parasitic strains showing drug resistance with the current therapeutic regimen and vaccine trials are still underway. Thus, there is a constant demand for a stable and effective antimalarial. In this context, apicoplast, a relict plastid in the *Plasmodium* is looked upon as a putative drug target due to its prokaryotic origin. It acts as a site for four major metabolic pathways which includes a prokaryotic type (Non-mevalonate) Isoprenoids biosynthesis or MEP pathway. All four pathways are hypothesized to be essential for the survival of the parasite. Majority of the enzymes involved in all these pathways are encoded by the parasite nuclear genome and are targeted to the apicoplast via a bipartite leader sequence. In the present study we have characterized two major enzymes IspD and IspG from *P. vivax* isoprenoid biosynthesis pathway. This pathway is responsible for the synthesis of isoprene units and is indispensible for the erythrocytic stages of the parasite. We have cloned and expressed PvIspD and PvIspG proteins from Indian field isolates. The proteins were purified and tested for their biochemical activity. The purified proteins were used to raise antibodies which were further used to co-localize the protein in the apicoplast of the parasite, indicating to the transcriptional activity of these proteins in the parasite. In-silico studies of proteins indicate the presence of conserved domains across all apicomplexans and prokaryotes. We have tested binding of different drugs with these enzymes and hypothesize a strong probability of using these enzymes as targets to inhibit erythrocytic stages of parasite.

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NOVEL CLUSTERING ALGORITHM IMPROVES HAPLOTYPE DETERMINATION FROM PACBIO TARGETED AMPLICON SEQUENCING DATA

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Next-generation sequencing of targeted PCR amplicons provides improved understanding of microbial population structures such as those found in *Plasmodium* infections. While Ion Torrent and Illumina are regularly used for amplicon sequencing, these technologies are limited to amplicons less than 500 bp. Longer amplicons and read lengths can capture more complete haplotypes and transverse highly variable or repetitive regions. Technologies such as PacBio circular consensus sequencing can generate sequences kilobases in length, but suffer from higher error rates, which make analysis difficult when attempting fine resolution in determining haplotypes (e.g. detecting one base pair difference). Several approaches have been used to help correct for these errors including using Illumina in conjunction with PacBio to help correct the error prone long reads as well as several clustering and data curation methods. However, de novo assembly and clustering of long haplotypes remains a challenge. We have developed a novel clustering algorithm to mitigate the error rates of PacBio without the aid of Illumina. This algorithm outperforms current clustering methods and will be integrated into our SeekDeep software suite (baileylab.umassmed.edu/SeekDeep). The algorithm is based on rapidly creating clusters using kmer distance between sequences. Once

sequences have been clustered, a consensus sequence for each cluster is generated creating a high-fidelity haplotype. We have tested the algorithm on mixtures of *P. falciparum* lab strains from 1.6kb and 3kb fragments of the VAR2CSA gene and detected all known haplotypes perfectly down to 1% abundance. The algorithm has performed well on clinical samples including the 1.6kb VAR2CSA *P. falciparum* fragment and the protein sequences of *P. vivax* CRT and *P. vivax* CSP, both of which contain repetitive regions. Thus, our new algorithm provides researchers with the ability to further detail and define the diversity within microbial and parasitic populations.

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EVIDENCE OF SELECTION AND GENE COPY NUMBER VARIATIONS IN VIRULENCE FACTORS AND RESISTANCE GENES IN *PLASMODIUM FALCIPARUM* FROM LORETO - PERU

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Malaria is affects more than 300 million people causing more than 2 million deaths per year. Of the four *Plasmodium* species known to infect humans, *P. falciparum* stands out as the most severe. There is increasing evidence that the emerge of resistance to anti-malarial drugs, migration and limited access to adequate health care contribute to the spread of malaria in endemic regions. This data underscores the need of constant surveillance in these settings. In this study, we analyzed the genomes of 16 *P. falciparum* strains isolated from the endemic Amazonian region of Loreto in Peru during a high incidence period between 2004 to 2008. For this purpose, we conducted a genomic population analysis focused on the detection of SNPs, gene copy number variations and selection. Our results identified the presence of four subpopulations with a very recent origin in the region with evidence of gene flow between them and presence of polyclonal infections. Read depth analysis detected five clusters with increased gene copy numbers in chromosomes 4,5,10,11 and 12 that affected genes with roles in invasion and immune evasion like reticulocyte binding proteins, MSPs, duffy binding-like merozoite surface protein and others. Additionally, we found increased gene copy number in GCH1 in five isolates and MSP1, MSP2 and VP2 in one single isolate. The genomic analysis of neutrality employing TajimasD and FuFst identified two genomic regions under balancing selection. Genes located in these regions include reticulocyte binding proteins, erythrocyte membrane protein and SURFIN. The finding of increased copy number in genes mediating host-parasite interaction can result in different expression profiles potentially influencing parasite development and severity of certain strains. Increase in copy number in genes associated with resistance pose a potential threat to anti-malarial drugs employed in this setting and underscore the need to track the origin of the VP2 expansion. Finally, the evidence of selection in genes mediating invasion point towards the effect of immune pressure in positive selection on diversity.

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INFLUENCE OF SEASONAL MALARIA CHEMOPREVENTION ON MARKERS OF T CELL EXHAUSTION AND IMMUNOREGULATION

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In addition to causing acute and sometimes fatal illnesses, *Plasmodium falciparum* infections may also compromise immune responses to some vaccines, and thus antimalarial treatments have been considered in conjunction with vaccination. Here, in the context of Seasonal Malaria Chemoprevention (SMC), we examined whether malaria prevention decreases the frequency of exhausted T cells and regulatory T cells. The study was conducted in two villages in the district of Ouelessebougou in Mali, an area of intense seasonal transmission. Children either received SMC (Beneko village) or not (Ferekoroba village), and were followed for 6 months with monthly assessment of T cell markers in *ex vivo* assays. In the group that received SMC, 42% of children (21/50) remained free of *P. falciparum* (as assessed by blood smears) during the follow-up period compared to only 16% (8/50) in children who did not receive SMC. Despite the impressive effect of SMC treatment on blood stage parasitemia rates, there was no discernible effect on the level of exhausted T cells as measured by the expression of PD-1, LAG3 and CD160. The levels of T regulatory cells (CD4+FOXP3+ T cells) were increased in the non-SMC group and in a subset of the SMC group that were infected. We speculate that under intense malaria transmission, frequent antimalarial treatment (45/50 children in the group that did not receive SMC and 28/50 in the group that received SMC) complicates the ability to observe the effect of SMC on the immune system. We are currently evaluating the effect of malaria prevention during the low transmission season when malaria incidence and treatment is low, and we will present these data for comparison.

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SEASONAL MALARIA CHEMOPREVENTION IS ASSOCIATED WITH A REDUCTION IN SEROPOSITIVITY TO BLOOD-STAGE ANTIGENS

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Seasonal Malaria Chemoprevention (SMC) entails 3-4 therapeutic doses of antimalarials given to children at monthly intervals. SMC was approved by WHO in March 2012 for malaria control in countries with seasonal malaria transmission, and implementation is being scaled up in Mali. While SMC has substantial benefits against malaria infection and disease, the long-term impact on immunity to malaria is not well understood. We conducted cross sectional surveys at the beginning (N=579) and at the end (N=559) of the 2014 transmission season. Antibody levels to *Plasmodium falciparum* antigens MSP-142, AMA-1 and CSP were measured by ELISA.

Seropositivity was defined as OD above the mean plus three standard deviations of naïve donors. *P. falciparum* infection was diagnosed by blood smear. In August 2014, 53.4% of the children were infected prior to first SMC dose, compared to 22.2% in November one month after last dose. Seropositivity rates prior to and after dosing among uninfected children were 85.9% vs 73.6% to MSP-142, p<0.001; 42.2% vs 30.3% to AMA-1, p=0.001; and 19.4% vs 15.2% to CSP, p=0.2. Among *P. falciparum*-infected children, the rates prior to and after SMCwere 95.1% vs 91.1% to MSP-142, p=0.1; 54.0% vs 43.3% to AMA-1, p=0.05; and 30.4% vs 25.0% to CSP, p=0.3. The lower seropositivity rates at the end of the malaria season among children who received 3 monthly doses of SMC suggest that the reduced exposure to blood stage parasites is reducing immune recognition of blood stage antigens like AMA-1 and MSP-1. We are currently evaluating the effect of SMC given over 2 or 3 years on seroreactivity rates to blood stage and preerythrocytic malaria antigens.

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IDENTIFICATION OF IMMUNE SIGNATURES UNDERLYING CLINICAL IMMUNITY TO *PLASMODIUM FALCIPARUM* MALARIA

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Naturally acquired immunity to malaria is a complex and poorly understood process, with increasing evidence pointing towards an orchestration of multiple immune mechanisms triggered by a highly diverse set of antigens. Various studies have shown how the acquisition of antibodies against individual or subsets of surface antigens correlate with increased protection against clinical malaria in an exposure and/or age-dependent manner. Nevertheless, inconsistent and sometimes contradictory results raise the question if the observed correlates are indeed the functional components of a protective immune response or to what degree they proxy previous exposure and are indicative of other underlying immune mechanisms. Furthermore, limited efforts have so far been made in relating these immune correlates to a particular degree of individual-level protection. Here we analysed cohort-based, *P. falciparum*-specific antibody profiles using a machine learning approach to identify immune signatures predictive of an individual's protective status against clinical infection. We demonstrate that due to small effect sizes, antibody responses against commonly assumed immune correlates and potential vaccine targets are poor predictors of clinical immunity. On the other hand, highly predictive immune signatures can be found in data incorporating a much broader set of internal and surface-expressed parasite proteins. Models build on these signatures show a high degree of accuracy in predicting individual-level protection against symptomatic infections during the next transmission season. Our results suggest that naturally acquired protection is the consequence of a focused accumulation of responses to a variety of both conserved and polymorphic antigens but with no single antigen offering high-level protection on its own.

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EFFECT OF ALLELIC POLYMORPHISM ON MALARIA PARASITE SPECIFIC *EX VIVO* IFN-GAMMA (IFN-γ) RESPONSES TO APICAL MEMBRANE ANTIGEN 1 (AMA1) IN A MALARIA EXPOSED POPULATION

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One of the leading causes of morbidity and mortality, especially in children in Africa, has been malaria caused by *Plasmodium falciparum* species. However, the delivery of core malaria interventions is responsible for the percentage decrease in malaria death rate since the year 2000. But the global technical strategy and targets for malaria 2016-2030 cannot be attained with these interventions because, malaria has not been controlled by widespread deployment of existing control tools in many endemic areas. An effective vaccine would complement these efforts. Effective anti-malaria vaccine will likely require vaccine constructs designed to induce protective CD8+ T cells against malaria liver stages. The leading malaria vaccine candidate antigen expressed by sporozoite, liver and blood stage parasites is the *P. falciparum* Apical Membrane Antigen-1(AMA1), but its allelic polymorphism is a stumbling block for vaccine development. Limited data exists on the effect of AMA1 polymorphisms on T cell responses. This study is therefore designed to investigate the effect of allelic polymorphism on malaria parasite specific *ex vivo* IFN-γ response to AMA1 in a malaria exposed population. Five study volunteers with the following HLA A and/or B super types (A01, B27, B07, B58, A02, A01A03, A03, B44, and A03) were selected from a previous study. Bioinformatically selected MHC class I-specific AMA1 epitopes from 3D7 strain, were aligned with sequences from 7G8, FVO, tm284, FC27 and AAN35928 AMA1 strains for selection and synthesis of peptides with variability. A total of 133 peptides were selected and synthesized, for stimulation of volunteers' peripheral blood mononuclear cells in IFN-γ Enzyme Linked Immunospot Assay (ELISpot). The Fisher's exact test will be used to compare proportions of IFN- γ responders between the different allelic forms of AMA1. Pairwise post hoc test for differences in proportions will be performed if statistically significant differences are observed in proportions of positive responders to the various antigens.

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CONTRIBUTION OF PARASITE AND HOST DIVERSITY TO MALARIA TRANSMISSION IN GHANA

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The transmission of malaria parasites depends on the presence of the sexual stages (gametocytes) of *Plasmodium falciparum* in the blood. The probability of establishing an infection in the mosquito depends on several factors including the densities of male and female gametocytes as well as their sex ratio. This then makes it interesting to determine how gametocytes are rendered infectious as well as understand the dynamics of the development of transmission blocking antibodies which are developed against these gametocytes and also better understand how these work together to cause malaria trasnmission. We intend to i)- compare the prevalence of gametocytes and transmission blocking antibodies against Pfs48/45 and Pfs230 and ii)- determine the diversity of factors such as cytokines which also influence malaria transmission in two areas with different malaria transmission intensities. Blood samples were collected from *P. falciparum* infected and uninfected patients living in two different malaria transmission intensity. Total IgG (and subclasses IgG1 and IgG3), IgM, IgE levels were determined by ELISA. The levels of cytokines in the plasma (TNF, INF-γ and IL-10) were estimated by Multiplex. Gametocyte prevalence was measured by microscopy and qRT-PCR. Highly variable seroprevalence of antibody responses against sexual stage parasite antigens was found. A marked prevalence and significantly higher level of antibodies was found in volunteers from the high malaria transmission intensity. However the cytokines profiling is negatively correlated to antibody level. But these results are preliminary we expect to have more before the meeting and better interpreted.

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PURIFICATION OF *PLASMODIUM* SPOROZOITES ENHANCES PARASITE-SPECIFIC CD8+ T CELL RESPONSES

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Malaria infection caused by *Plasmodium* parasites continues to cause enormous morbidity and mortality in endemic populations, and there is no licensed vaccine capable of inducing sterile protection. Hyperimmunization with attenuated whole sporozoites can induce sterily protective immune responses targeting pre-erythrocytic antigens. Most animal models of hyperimmunization rely on sporozoites dissected from

mosquito salivary glands and injected without further purification. In BALB/c mice, repeated small doses of P. yoelii sporozoites progressively expand the population of sporozoite-specific CD8+ T cells. In this study, large secondary doses of unpurified sporozoites unexpectedly led to contraction of sporozoite-specific CD8+ T cell responses in sporozoite-primed mice. While sporozoite-primed CD8+ T cells can alternatively be expanded by secondary exposure to Listeria monocytogenes expressing recombinant *Plasmodium* antigens, such expansion was potently inhibited by co-injection of large doses of unpurified sporozoites and by uninfected salivary glands alone. Purification of sporozoites away from mosquito salivary gland debris by density gradient centrifugation eliminated salivary gland-associated inhibition. Thus, the inhibitory effect appears to be due to exposure to uninfected mosquito salivary glands rather than sporozoites. To further assess the effect of salivary gland exposure on later sporozoite vaccinations, mice were immunized with uninfected salivary glands from a single mosquito. Compared to naïve mice, salivary gland pre-sensitization reduced subsequent liver burdens by 71%. These data show that component(s) in mosquito salivary glands reduce liver infection, thereby limiting antigen dose and contributing to lower magnitude T cell responses. These findings suggest that sporozoite immunogenicity studies be performed using purified sporozoites whenever feasible.

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T-CELL DYNAMICS REVEAL A POTENTIAL ROLE FOR CD8+ T-CELLS DURING BLOOD-STAGE *PLASMODIUM CYNOMOLGI* INFECTION OF RHESUS MACAQUES

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T-cells play a critical role in malaria immunology. They are important for the development of protective immunity against the parasite but can cause immunopathology when over-exuberant or dysregulated. The roles of CD4+ T-cells are well-appreciated in these processes, but the role of CD8+ T-cells during blood-stage malaria has received less attention. Most studies to-date have focused on studying T-cell responses against *Plasmodium falciparum* or mouse models of malaria. Contrastingly, T-cell responses during *P. vivax* infection are less understood, in part due to the lack of appropriate and tractable animal models for this malaria parasite. In this study, the *P. cynomolgi* –rhesus macaque model of *P. vivax* infection was used to monitor the T-cell dynamics during primary and relapse blood-stage infections. Immunophenotyping of T-cells during acute infection revealed a decrease in the total number of T-cells compared to pre-infection values as expected based on previous experiments. Although both CD4+ and CD8+ T-cell numbers decreased at this point, the CD8+ compartment was the most affected. Interestingly, the loss of CD8+ T-cells in the periphery appeared to correlate with clinical presentation. In agreement with the flow cytometry data, whole-blood transcriptomic analysis also indicated that the peripheral T-cell compartment, in particular CD8+ T-cells, was altered during acute infection. These results corroborate previous findings showing decrease in CD8+ T-cell subsets in *P. vivax*-infected patients. Interestingly, the transcription of IL-15 and Granzyme B was decreased in peripheral cells but this did not correlate with protein levels that were increased in the plasma. Collectively, these data suggest that activated Granzyme B-secreting CD8+T cells, although reduced in number in the circulation, may be homing to other organs and releasing inflammatory mediators during the infection. Overall, these data indicate that CD8+ T-cells are involved in blood-stage *P. cynomolgi* infection and may be related to disease severity.

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DECREASED MALARIA TRANSMISSION IN KENYA LED TO DELAYED ACQUISITION OF ANTI-MALARIAL ANTIBODIES IN CHILDREN AND ADULTS

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Malaria transmission is declining worldwide due to interventions such as insecticidal treated bed nets and indoor residual spraying. In our study population in western Kenya, we have observed a decline in the prevalence of asymptomatic *Plasmodium falciparum* (Pf) infection in children from 81% in 2003 to 14% in 2013. It has long been known that antibodies play an important role in the development of immunity to malaria. We sought to analyze changes in acquisition of anti-malarial antibodies over a 10-year period of declining transmission. Bioplex was used to measure immunoglobulin (Ig) G antibodies specific for 34 malarial proteins in healthy Kenyan children and adults from two cross-sectional cohorts (n=82 children, n=95 adults in 2003, and n=97 children, n=50 adults in 2013). The antibody magnitudes, prevalence, and seroconversion rates were directly compared between the two cohorts (2003 and 2013). Compared to the 2013 cohort, children and adults in 2003 had higher antibody magnitudes and prevalence, and faster seroconversion rates for the majority of the antibodies measured. For example, in 2003 the age groups 1-3, 4-6, 7-10, and 18+ years had prevalence values for merozoite surface protein (MSP) 6 of 50.0%, 58.3%, 83.3%, and 88.4%; in 2013 prevalence values for the same age groups were 17.2%, 27.0%, 32.3%, and 68.0%, respectively. Antibody magnitudes and prevalence in both cohorts tended to increase with age, as expected. An exception to this pattern was seen in antibodies to 4 out of 5 domains of Pf Erythrocyte Membrane Protein 1 (PfEMP1), in which children in 2003 and had higher antibody magnitudes than adults in 2003, while in 2013 antibody magnitudes increased with age. In summary, decreasing Pf transmission is associated with a decline in several anti-Pf antibodies across age groups. Lower transmission rates may lead to a slower acquisition of anti-malarial immunity and longer duration of susceptibility to symptomatic Pf infections.

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ANTIBODY RESPONSES IN MALARIA-NAÏVE ADULTS AFTER IMMUNIZATION VIA MOSQUITO BITE WITH RADIATION-ATTENUATED *PLASMODIUM FALCIPARUM* SPOROZOITES (IMRAS)

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Immunization with radiation-attenuated *Plasmodium falciparum* sporozoites (PfRAS) via mosquito bite serves as the gold standard for protective immunity against malaria and provides a relevant model for studying protective immune effector mechanisms. We recently completed a phase 1 clinical trial (Immunization via Mosquito bite with Radiation-Attenuated Sporozoites, or IMRAS), a comprehensive systems biology-based effort to identify biomarkers of protection, including host response and antigenic targets, by comparing protected, non-protected, and mock-immunized subjects. There is evidence that humoral immunity

contributes to RAS-induced protection in that immune serum from RAS-immunized humans can block the invasion of Pf sporozoites into hepatocytes. In addition, high titers of CSP-specific antibodies in humans correlate with protection against infectious challenge. In the present study, we investigated the generation of malaria-specific antibody responses in PfRAS-immunized subjects, and assessed their associations with sterile protection from controlled human malaria infection (CHMI). Serum IgG antibodies specific for pre-erythrocytic antigens (CSP, AMA-1, and CelTOS) were quantified by the International Reference Center for Malaria Serology Laboratory (WRAIR) using validated ELISA assays at the following time points: pre-immunization, day 14 post 3rd immunization, day of CHMI and day 28 post CHMI. In addition, sporozoite-specific antibodies were measured by an immunofluorescence antibody (IFA) assay using imaging analysis software at the pre-immunization and day of CHMI time points. Results from this study will be integrated with those from current and future studies with IMRAS samples using advanced immunobiology systems analysis to identify immune signatures of protection elicited by whole sporozoite vaccination.

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IDENTIFICATION OF PFEMP1 EPITOPES USING A DIVERSITY-COVERING ULTRADENSE PEPTIDE MICROARRAY

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Plasmodium falciparum erythrocyte membrane protein-1 (PfEMP1) antigens play an important role in parasite sequestration and host immune system evasion. PfEMP1s are encoded by the extraordinarily diverse *var* gene family, present in 40 to 60 copies per genome. Acquired antimalarial immunity is at least partially due to antibodies directed against highly variable antigens like PfEMP1 that are present on the red blood cell surface. While we have previously identified PfEMP1 fragments whose seroreactivity is associated with increased malaria exposure and thus potential immunity, the critical PfEMP1 epitopes that drive such immune-mediated protection have not yet been identified. A peptide microarray is a tool that, with carefully chosen serum samples, allows for recognition of peptides that may harbor epitopes critical for natural protection against malaria. We designed a pilot PfEMP1 peptide array to identify such PfEMP1 epitopes. Eighty-three thousand 16-amino acid (aa) peptides were spotted onto an array, spanning the complete *var* repertoires of the reference genome 3D7, the Indochina strain DD2, and an Indian isolate, RAJ116, with 12-aa overlap. This included a total of 151 PfEMP1s, providing coverage of both extracellular and intracellular PfEMP1s and all previously described PfEMP1 domain cassettes. We probed this array with sera from children aged 1-6 years old and adults from rural Mali. Among extracellular and intracellular PfEMP1 fragments recognized more intensely by adults than children, potentially representing an effective immune response, we identified key peptides associated with this seroreactivity. We also identify such peptides in domain cassettes associated with severe malaria. We anticipate that this approach will allow targeted identification of specific regions in PfEMP1 constitutive domains that harbor epitopes critical to natural immunity. We present plans for future work with this powerful new tool.

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AN ULTRA-DENSE PEPTIDE ARRAY FOR IDENTIFYING HUMAN ANTIBODY BINDING SITES ON MALARIA PARASITE ANTIGENS

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A high-throughput means of identifying epitopes involved in antibody recognition of malaria antigens would heighten our understanding of natural immunity to malaria. Previous epitope mapping approaches do not enable simultaneous study of multiple antigens or antigenic variants. We designed an ultra-dense peptide array containing multiple *P. falciparum* antigens and field sample-derived diverse variants. Arrays were fabricated *in situ* using light-directed maskless array synthesis. We included vaccine candidate antigens, gametocyte antigens, variant surface antigens, and mosquito salivary antigens to inform vaccine design, development of serosurveillance assays, transmission capacity, naturally acquired immunity, and species-specific vector exposure. 172,396 peptides representing 209 antigens were included. Sequences were derived from Sanger and genome sequencing of samples from Mali, including those from a phase II pediatric vaccine study, and Southeast Asian samples from an artemisinin resistance study. PfEMP1s (n=151) were included as 16-amino acid (aa) peptides overlapping by 12-aa (16/12 overlap). Other antigens were included in triplicate with 16/12 overlap; a subset was additionally included with 16/15 overlap. The most diverse antigen, AMA1, had 331 variants (14,153 non-redundant peptides). PfEMP1s represented the greatest proportion of the array with 83,760 peptides. Reactivity of sera extracted from dried blood spots (DBS) on Whatman® 903 cards and from whole blood was highly correlated, albeit with some loss of dynamic range for DBS samples, thus justifying future field sample collection without a cold chain. Sera collected from Mali and Southeast Asia recognized previously described and novel epitopes in pre-erythrocytic and blood stage antigens including CSP, AMA1, and variant surface antigens. We identify antigens and peptides underlying the higher reactivity of adult sera as compared with pediatric sera. With this approach, an ultra-dense peptide array illuminates acquisition of natural immunity to malaria and can provide insights applicable to vaccine design and serosurveillance.

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THE TRANSCRIPTION FACTOR T-BET COMPROMISES HUMORAL IMMUNITY TO BLOOD-STAGE MALARIA BY INHIBITING THE EFFICIENT DEVELOPMENT OF GERMINAL CENTRE RESPONSES

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Naturally acquired immunity to malaria develops only after many years of repeated exposure to *Plasmodium* parasites. Despite the key role that antibodies play in protection against malaria, the cellular processes underlying the slow acquisition of immunity are unclear. Children in high transmission settings that experience frequent malaria clinical episodes are characterized by a delayed development of parasite-specific memory B cells, suggesting that the inflammatory factors contributing to disease

hinder these responses. We have recently addressed this hypothesis using a pre-clinical model of severe malaria and found that the same inflammatory pathways mediating disease syndromes impair T follicular helper cell differentiation and the development of germinal centre (GC) responses required for antibody-mediated control of parasitemia. To further define the impact of inflammation in the induction of protective immunity, the development of GC responses to *P. berghei* ANKA was examined in mice deficient in the pro-inflammatory transcription factor T-bet. Genetic deletion of T-bet significantly improved T follicular helper cell differentiation rates, which translated in enhanced GC and parasite-specific antibody responses to infection. Infection of T-bet$^{fl/fl}$CD23^{Cre} mice, with specific deletion of T-bet in their B cell compartment revealed that antibody production and isotype-switching was also regulated by B-cell-intrinsic expression of T-bet. Moreover, the induction of GC B cells and total plasma cell responses to infection were significantly improved in the absence of T-bet expression specifically in B cells. Thus these data suggest that inflammatory pathways elicited in response to clinical malaria negatively impact the development of long-term humoral immunity not only by inhibiting T cell help for antibody formation but also by directly modulating B cell responses to infection.

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REDUCED *PLASMODIUM* BURDEN IN HUMANS ASSOCIATES WITH CD38+ CD4+ T CELLS DISPLAYING CYTOLYTIC POTENTIAL

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Malaria is associated with complex multi-factorial immune responses, and the exact molecular mechanisms required to control parasite burden remain largely unknown. The recent discipline of systems immunology integrates immunology with molecular and computational sciences to enable comprehensive and quantitative evaluation of human immune responses at a level of detail previously restricted to murine models. We are applying systems immunology approaches to characterize the host response to the *Plasmodium spp* parasite in humans. Using a unique resource of samples from a controlled human malaria infection study, we identified a novel population of CD4$^+$ T cells whose frequency in peripheral blood was inversely correlated with parasite burden following *P. falciparum* infection. These CD4$^+$ T cells expressed the multifunctional ectoenzyme CD38 and had unique features distinguishing them from other CD4$^+$ T cells. Specifically, their phenotype was associated with proliferation, activation and cytotoxic potential as well as significantly impaired production of IFN-γ and other cytokines and reduced basal levels of activated STAT1. A CD38$^+$ CD4$^+$ T cell population with similar features was identified in healthy uninfected individuals, at lower frequency. This is the first report of a population of CD4$^+$ T cells with a cytotoxic phenotype and markedly impaired IFN-γ capacity. The expansion of this population following parasite infection and their ubiquitous presence in humans suggests that they may have a broad role in host-pathogen defense.

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HOST IMMUNITY TO MALARIA INFECTION, ANAEMIA AND SOCIO-ECONOMIC IMPACT AMONG CHILDREN LESS THAN 10 YEARS IN NORTHERN CAMEROON

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Malaria and anaemia are key public-health challenges in Cameroon. However, little has been reported on the interaction between these interconnected health determinants. The present study was designed to investigate the relationship between malaria occurrence, immunity, anaemia and socio-economic impact among under - ten children living in an area of intense seasonal malaria transmission in Northern Cameroon. A Cross- sectional survey was conducted in November 2013, in Pitoa and Mayo-Oulo Health Districts, Northern Cameroon. Total, 368 children aged 6months - 10 years were recruited. Finger-prick blood samples collected were used for haematocrit; immunoglobulin gamma level determination using ELISA; malaria parasite prevalence, specie and density by microscopy; *Plasmodium* DNA extraction from filter paper for PCR. A structured questionnaire was used to assess Socio-economic status.Data analysis was by SPSS 20.Overall prevalence of malaria and anaemia were 32.9% and 20.6% respectively. Globally, 46.4% of the children (95% CI: 41.1 - 51.8) were low anti-malarial Total IgG producers, 36.2% (95% CI: 31.2 - 41.5) low IgG1 producers and 19.8% (95% CI: 15.7- 24.3) were low IgG3 producers. There was no statistically significant (p>0.05) association between immunity and malaria status for all the categories of IgG. The Socio-economic status of the population was poor. Malaria was not the cause of anaemia in the children. Therefore, other factors may have accounted for anaemia. Since no effect of malaria and immunity was observed in the low production of IgGs, the IgG levels observed could not be an indicator of any protection against malaria but may be due to humoral response to malaria infection.Malaria programmes should rapidly scale up on improving the health and immunity status of the anaemic in poor communities.Future studies should focus on finding out the causes of anaemia in malaria - infected children.

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PROFILES OF PFEMP1-SPECIFIC IGG ANTIBODIES FROM BIRTH TO 12 MONTHS OF AGE IN BENINESE INFANTS

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The cytoadherence properties of *Plasmodium falciparum* infected erythrocytes (IE) represent a major contributor to the pathogenesis of malaria through interactions with various endothelial cell surface receptors. These interactions are mediated by members of the highly variable *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) expressed on the IE surface. One particular component of PfEMP1 proteins, the cysteine-rich interdomain region (CIDR), is known to play a very important role in the adhesive interactions between IE and endothelial receptors, making this region a potential vaccine target of interest. Here, we investigated the dynamics of maternally-transferred IgG antibodies targeting the CIDR of a panel of different PfEMP1 proteins, as well as infants' own acquisition of antibodies with the same specificities during the first year of life. We used plasma samples collected longitudinally from the offspring of a cohort of pregnant women who had themselves been followed closely through pregnancy. We show that the levels of all anti-CIDR antibodies quantified declined to the point of disappearing over the 6 first months of life. Antibodies with specificity for the CIDR predicted to adhere to selected receptors (CD36, EPCR) or for the CIDR associated with the unknown phenomen were subsequently acquired by infants between 7-12 months of age, their levels being a function of *P. falciparum* history during infancy. Infected infants developed stronger antibody responses to the CIDR associated with either EPCR binding or unknown compared to uninfected infants. The transcriptional profile of var genes showed no obvious difference between parasites infecting the children before and after 6 months except for some genes of group B var.

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PLASMODIUM FALCIPARUM EPIDEMIOLOGY IS GOVERNED BY MULTI-SCALE IMMUNE SELECTION AND A DIVERSITY-TRANSMISSION FEEDBACK

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The human malaria parasite *Plasmodium falciparum* displays an extensive degree of antigenic diversity that facilitates long periods of infections and high re-infection rates. Of particular interest are the variant surface antigens (VSA) encoded by several multi-gene families, including the *var* genes whose protein products PfEMP1 are implied in malaria infection pathology and immune evasion. Within-host clonal antigenic variation of PfEMP1 limits host exposure to the parasite's antigen repertoire by the predominant and cyclic expression of single variants during infection. At the population level, high levels of antigenic diversity between parasites result in hosts acquiring protection against severe and symptomatic infections in a piecemeal process over years of repeated exposure. As antigenic diversity is predominantly generated through recombination it is expected to respond dynamically to changes in transmission and immune selection. On the other hand, population-level prevalence and incidence can be seen as a direct consequence of antigenic diversity, thus forming a non-linear feedback between transmission intensity and diversity. To explore this feedback and its consequences on malaria epidemiology in more detail we developed an individual-based, multi-scale modelling framework in which antigen diversity emerges as a dynamic property from the underlying transmission dynamics. New antigenic variants are generated through recombination and mutation during infection and the mosquito-stage and are subjected to within and between-host selection pressures. Our results show that immune selection severely limits the level of antigenic diversity that can be stably maintained at any given time and that the transmission-diversity feedback can significantly affect how population-level prevalence responds to changes in disease transmission due to natural fluctuation or intervention measures, which has important implications for our understanding of malaria epidemiology and disease control efforts.

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A PROSPECTIVE STUDY OF B CELL DYNAMICS IN PATIENTS WITH MALARIA USING MASS CYTOMETRY

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Clinical immunity to malaria is largely mediated by humoral immune responses. Protective immunity, however, appears to require repeated exposure and wane in the absence of continuous infection. It has been proposed that malaria impairs the function of B cells and contributes to the slow development and incomplete immunity observed in malaria. The mechanisms behind this are as of yet unclear. In the current study we have investigated the dynamics of B cell phenotypes at six time points over the course of one year, in patients treated for *Plasmodium falciparum* malaria at the Karolinska University Hospital in Stockholm. Study participants were stratified according to previous malaria exposure in order to compare immune responses of malaria-naïve individuals (n=3), with individuals from malaria-endemic areas (n=3). We present an extensive mass cytometry (CyTOF™) characterisation of B cell phenotypes. Our results demonstrate the existence of specific B cell subpopulations that arise at different time points and differently in individuals with previous malaria exposure.

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LONGITUDINAL ASSESSMENT OF PFSPZ-SPECIFIC T CELL RESPONSES IN MALARIA-NAÏVE ADULTS VACCINATED WITH PFSPZ VACCINE

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An attenuated *Plasmodium falciparum* (Pf) sporozoite vaccine (PfSPZ Vaccine) is being evaluated in humans for efficacy. Here we present T cell responses after vaccination with 9.0x10^5 PfSPZ administered 3 times at 8 week intervals to malaria-naïve adults. The phenotype and effector functions of Pf-specific memory CD4, CD8, and γδ T cells were assessed by 14-16-color flow cytometry throughout the course of vaccination. Pf-specificity was determined by incubating PBMCs with aseptic PfSPZ or the vaccine diluent (HSA). PfSPZ Vaccine induced high magnitude multi-functional IFNγ, IL-2, and/or TNFα cytokine-producing memory CD4 T cell responses. Surprisingly, these responses peaked at 1.5% (mean) after the 1st immunization, and declined to 0.57% and 0.51% after the 2nd and 3rd immunizations, respectively. PfSPZ-specific cytokine-producing memory CD8 T cell responses also peaked after the 1st immunization (0.39% mean) and declined to pre-vaccine levels after the 3rd immunization. The activation markers HLA-DR and CD38 on T cells were used to provide a sensitive measure of T cell activation *in vivo*. The frequency of HLA-DR+CD38+ memory CD4 T cells peaked two weeks after the 1st immunization, but returned to baseline (pre-vaccine) levels two weeks after the 2nd and 3rd immunizations. Last, the Vδ2+ sub-family of γδ T cells expanded 2.8-fold above pre-vaccine levels, and this increase persisted after all immunizations. γδ T cells showed a very high level of HLA-DR+CD38+ co-expression, peaking at a mean of 34% two weeks after the 2nd immunization, and declining after the 3rd immunization. In conclusion, PfSPZ Vaccine induced high frequency Pf-specific CD8, CD4, and γδ T cells after the first vaccination with 9.0x10^5 PfSPZ. The magnitude of Pf-specific CD8 and CD4 T cells was dramatically reduced after the 2nd and 3rd immunizations, suggesting that PfSPZ Vaccine induced rapid anti-PfSPZ immunity that limited subsequent boosting with PfSPZ Vaccine at the dose and interval tested here. Based on these data, it may be possible to achieve equivalent protective efficacy with fewer immunizations.

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ASSESSING THE IMPACT OF NON-ADHERENCE TO ANTIMALARIALS USING WITHIN-HOST MODELING OF FALCIPARUM MALARIA

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Artemisinin combination therapy regimens typically contain 3 or 6 doses spread over 3 days. This ensures that the blood concentration of the drugs remains high enough for a sustained period of time, reducing the probability that parasites survive and recrudesce. Taking the correct doses of antimalarials at the right time will maximise their clinical impact. The efficacy of ACTs is usually compared in clinical trials where there is a high level of patient adherence to the drug regimen, but adherence can be much lower in routine health care settings, and is likely to vary according to the complexity and length of the regimen, as well as side effects of the

drugs amongst other factors. Much information on patient adherence has been collected, but it is difficult to assess the clinical impact of incomplete adherence in research settings. In this work, we have developed a stochastic within-host model of asexual parasitaemia in individuals with no previous exposure to malaria, which we calibrated against data from malariatherapy patients. Combining this model with a pharmacokinetic-pharmacodynamic (PKPD) framework enables us to estimate potential levels of treatment failure following poor adherence to malaria treatment. We include variability between individuals both in terms of parasite dynamics and pharmacokinetics. We simulate treatment with artemether-lumefantrine (AL) and estimate the probability of treatment failure after taking different numbers of doses, or taking the doses at the wrong time, using values based on adherence studies. Our modeling structure also allows other drug combinations to be scrutinized in this way.

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FORECASTING MALARIA ADMISSIONS AT A RURAL DISTRICT HOSPITAL IN WESTERN KENYA USING REMOTE SENSING DATA

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In sub-Saharan Africa, malaria kills a child every two minutes. In response to still high incidence of malaria, WHO developed the new global technical strategy for malaria 2016-2030. One of the three pillars of this strategy is to use surveillance as a key intervention in malaria control and elimination. Malaria surveillance data provides opportunity to develop a malaria early warning system and tracking of progress towards elimination. Therefore, in this study we use malaria admission data of children under five years of age at Siaya district hospital in Western Kenya to develop models to forecast malaria admissions incorporating remotely sensed environmental data. Three models were developed assessing forecast accuracy at 1-month, 2-month and 3-month lead times. We used monthly totals of malaria admissions and precipitation. For Normalized Difference Vegetation Index and Land Surface Temperature we computed monthly means. The data covered the period from 2002 to 2013. Malaria admissions were modelled using a general additive model with a smooth function of time to capture trend; a cyclic cubic spline of month to capture seasonality; and splines of the environmental variables for each lead time. To adjust for residual autocorrelation, we incorporated autoregressive term of order 1 as random effect in the models. Malaria admissions were assumed to follow quasi-Poisson distribution adjusting for over dispersion. The period from 2002 to 2012 was used for model training and the year 2013 for model validation. The 1-month lead time model had the least Root Mean Squared Error for both the training and the test periods with values of 19.30 and 6.02 respectively. The 3-month model had the least mean absolute percentage error (MAPE) of 39.31% for the 2013 forecast values while 1-month lead time model had the least MAPE for the training period. The 3-month lead time model provided better forecast accuracy in terms of absolute differences between forecast and observed values for the 2013 malaria admissions. The 3-month lead time model can potentially be utilized as an early warning system allowing sufficient lead time for control activities.

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A QUANTITATIVE ANALYSIS OF THE IMPROBABILITY OF FERTILIZATION AT LOW GAMETOCYTEMIAS IN THE ABSENCE OF TRANSMISSION-ENHANCING MECHANISMS

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Successful infection of an *Anopheles* mosquito by *Plasmodium falciparum* gametocytes can occur over many orders of magnitude of gametocyte density in the human host, from levels that are not detectable with light microscopy to thousands of gametocytes per microliter of blood. The empirical curves for the success of infecting a mosquito as a function of gametocyte density are difficult to fit, as the success of infection at low gametocyte densities can be higher than expected, while the success of infection at high gametocyte densities rises slower than expected. Indeed, even at high densities, there is often incomplete infection success. We are particularly interested in the low-end of gametocyte densities because people carrying undetectable gametocytes contribute heavily to the infectious reservoir. At the low-end of densities, overdispersion of gametocytes in a bloodmeal partially helps to explain the observed success rate, but it is not the full story. Within the mosquito midgut, a male gamete searches a crowded environment in which there can be more than one million uninfected red blood cells per female gametocyte. We reexamine the sexual phase of malaria transmission from gametocytes in the human host up to gametes finding each other and fertilization occurring in the mosquito midgut. We construct a detailed analysis of the within-midgut dynamics and study the effects of male gamete swimming rate, gametocyte density, overdispersion of gametocytes per feed, clustering, and diuresis of the bloodmeal on the probability of successful infection at different human host gametocyte densities. Finally, we show residual under-prediction of success probability at low gametocyte density that could be explained by possible alternative mechanisms such as clustering or chemotaxis in the mosquito midgut and then provide estimates of the magnitude of these possible mechanisms based on observed success probability.

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A SIMULATION STUDY OF WHEN MALARIA CONTROL AND ELIMINATION PROGRAMS CAN SAFELY REDUCE VECTOR CONTROL EFFORTS

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Scale up of the coverage of malaria vector control interventions has led to significant reductions in malaria disease burden globally. Due to the considerable resources currently devoted to vector control, there is a need to determine if this expenditure should be sustained after significant reductions in transmission. We use a simulation model of malaria epidemiology and immunology (OpenMalaria) to predict malaria transmission and disease outcomes after stopping the deployment of vector control interventions under various settings. We conduct regression analysis of simulation results to derive predicted probabilities of resurgence, severity of resurgence and time to resurgence under scenarios defined by the pre-intervention entomological inoculation rate (EIR), case management coverage, and vector control coverage, amongst other parameters. Results indicate that, in the absence of secular changes in the underlying determinants of transmission (historically called receptivity), there are few scenarios under which vector control can be removed without a strong expectation of resurgence. These, potentially safe, scenarios are characterized by low historic EIR, successful vector control programs that achieve elimination or near elimination, and effective surveillance systems with high coverage and effective treatment of malaria cases. Programs and funding agencies considering scaling back or

withdrawing vector control from previously malaria endemic areas need to first carefully consider current receptivity and other available interventions in a risk assessment. Surveillance for resurgence needs to be continuously conducted over a long period of time in order to ensure a rapid response should vector control be withdrawn.

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EPIDEMIA: AN ONLINE PLATFORM FOR DATA ACQUISITION, INTEGRATION, AND ANALYSIS TO SUPPORT ECOLOGICAL FORECASTING OF MALARIA OUTBREAKS

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Malaria early detection and early warning systems are important tools for public health decision makers. Here we present technical information on the design and implementation of the EPIDEMIA online platform which serves as an automated tool for forecasting and early detection of malaria outbreaks in the Amhara Region of Ethiopia. The platform uses freely available, community-driven open source software including the R language for statistical computing, MySQL and PostgreSQL for relational data storage, and PHP, HTML, and CSS for web front-end. The R language was chosen because it is widely accessible and allows for the use of advanced statistical modeling techniques. As a result of these choices, the EPIDEMIA data platform can easily be modified to incorporate potential future statistical models, applied to other geographic areas, or applied to other diseases linked to environmental conditions. The EPIDEMIA platform's epidemiological data acquisition subsystem consists of a securely hosted and encrypted website where public health collaborators in Amhara upload malaria morbidity data which is then parsed and stored in a MySQL database. The environmental data acquisition subsystem uses our previously-developed EASTWeb software to continuously monitor, download, and summarize earth-observation data derived from satellite remote-sensing products that are freely-available on the internet. When new environmental data has been processed in EASTWeb's PostgreSQL database, a listener triggers a PHP script to import the new data into EPIDEMIA's MySQL database. The data integration subsystem, which is a series of scripted actions written in PHP, R, and MySQL stored procedures, integrates the various datasets. After each weekly upload of epidemiological data, the forecasting subsystem uses dynamic linear models implemented in R to analyze the integrated dataset for early signs of malaria outbreaks and to generate malaria forecasts. The reporting subsystem uses R to generate a weekly malaria forecast in PDF format and allows users to generate custom reports, visualizations, and data summaries using a query interface on the website.

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MATHEMATICAL MODELLING OF TAFENOQUINE FOR *PLASMODIUM FALCIPARUM* MALARIA ELIMINATION

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The 8-aminoquinolines are the only known class of antimalarials with action against *Plasmodium vivax* hypnozoites and *P. falciparum* gametocytes. Primaquine is widely used as part of treatment of *P. vivax* malaria to clear hypnozoites from the liver and thus prevent relapses. This requires a 14-day course to be taken. Primaquine has also been recommended by WHO since 2012 as a single dose for *P. falciparum* infection to kill gametocytes and thus interrupt transmission. A recent mathematical modelling consensus exercise for WHO to predict the efficacy of mass drug administration (MDA) for *P. falciparum* elimination found the additional impact of single dose primaquine to MDA with artemisinin combination therapy (ACT) to be very small. Tafenoquine is an investigational 8-aminoquinoline antimalarial currently being trialled as a hypnozoiticidal for treatment of *P. vivax* infection. Its mode of action and side effect profile are very similar to primaquine however it has the advantage of a much longer half-life. It has thus been proposed as a single dose alternative to 14 days of primaquine for *P. vivax*. However, tafenoquine is not currently being considered as a gametocytocidal for *P. falciparum* infection. In this role, its long half-life may confer greater transmission blocking activity than primaquine which could make it a valuable tool for falciparum elimination. In the absence of trial evidence, a mathematical model was developed to predict the efficacy of single dose tafenoquine alone and when given with ACT for *P. falciparum* malaria elimination. Using this model, a range of scenarios is being explored including its use for treatment of clinical cases, in mass drug administration, and as part of a combined strategy with vector control measures. In addition, its impact in low, medium and high transmission settings in Asia and Africa and for the elimination of artemisinin and ACT partner drug-resistant falciparum malaria. The results of this mathematical modelling exercise will be presented with recommendations for the possible role of tafenoquine in *P. falciparum* elimination and for potential clinical study designs to assess its efficacy in the field.

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IDENTIFYING MALARIA RISK FACTORS IN A HYPERENDEMIC SETTING USING BAYESIAN MODEL SELECTION

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The epidemiological dynamics of malaria, as with many other vector-borne diseases, have been linked to a wide variety of environmental, socio-economic, and demographic factors. Traditional statistical approaches for evaluating the contribution of each of these potential disease drivers present critical tradeoffs. Modeling all possible combinations can be computationally intensive and make it difficult to draw definitive conclusions when numerous disease factors are considered. Conversely, selecting a subset of potential drivers can fail to describe the relative importance of a particular covariate and can exclude important risk factors. To address these issues we propose a Bayesian probit regression model that contains a model selection procedure which proposes new candidate models through the random addition, subtraction, or swapping of covariates. A new model is proposed and evaluated at each step of the iterative Markov Chain Monte Carlo algorithm, generating parameter estimates and inclusion frequencies for each potential disease driver. We used this approach to simultaneously evaluate the relative importance of a wide range of environmental, socio-economic, and medical risk factors for malaria in the Bunkpurugu-Yunyoo district of northern Ghana, using existing data from six malaria surveys conducted in 2010-13. Our analysis identifies substantial protective socio-economic and medical factors related to the two modest "urban" centers in this small geographic area, indicating that the small towns in this hyperendemic setting may buffer nearby rural areas from environmental conditions that are traditionally linked to high malaria transmission. This Bayesian model selection technique offers a promising solution for dealing with the practical and computational constraints of evaluating numerous diverse risk factors for malaria and other diseases.

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ATTACKING THE MOSQUITO ON MULTIPLE FRONTS: INSIGHTS ON OPTIMAL COMBINATIONS OF VECTOR CONTROL INTERVENTIONS FOR MALARIA ELIMINATION FROM A MATHEMATICAL MODEL

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Despite great achievements by long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS), research demonstrates that these tools are insufficient to eliminate malaria transmission in many settings today. Protective coverage from these interventions is attenuated where mosquitoes can access blood resources from non-human hosts or from humans when they are outdoors. Fortunately, field experiments indicate that there are many promising vector control interventions that can be used to complement LLINs and/or IRS by targeting a wide range of biological and environmental mosquito resources. The majority of these experiments were performed to test a single vector control intervention in isolation; however, there is growing evidence and consensus that effective vector control will require a combination of interventions tailored to the target ecological and epidemiological setting. We present a mathematical modeling framework designed to examine combination interventions prior to empirical field trials. The model framework incorporates all stages of the mosquito life cycle from egg, larva, pupa, adult, and, crucially, the female gonotrophic cycle whereby females blood feed and lay eggs. We describe how the framework may be used to evaluate the impact of combining existing and novel interventions in synergistic ways in areas where LLINs and/or IRS are widely used but where malaria transmission persists. We consider the following vector control interventions in addition to LLINs and IRS: larvaciding (conventional and aerial), attractive toxic sugar baits (ATSBs), insecticide spraying of male mating swarms, mosquito-proofed housing, spatial and topical mosquito repellents, systemic and topical insecticide-treatment of cattle, odor-baited traps and space spraying. We describe optimal combinations of these interventions needed to significantly reduce entomological inoculation rate (EIR), a widely accepted measure of malaria transmission, in a range of ecological and epidemiological settings.

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IMPACT OF SEASONAL MALARIA CHEMOPROPHYLAXIS IN A HIGH AND SEASONAL MALARIA TRANSMISSION SETTING IN BURKINA FASO

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Seasonal malaria chemoprophylaxis (SMC) is a strategy endorsed by the WHO to prevent malaria in children aged 3-59 months living in seasonal malaria transmission areas. In Burkina Faso where malaria transmission is highly endemic and seasonal, more than 60% of malaria cases (clinical malaria) occur during the rainy season from June to October with the majority falling on younger children. In 2015, the SMC campaign in Burkina aimed at giving sulphadoxine-pyrimethamine and amodiaquine anti-malarial treatments (SP+AQ) to children below 5 years at one month interval during the rainy season. At each time point, children were treated with one dosage regimen (three unit doses over three days). About 8,000 community health workers were recruited to administer the SMC preventive treatment to about 650,000 children across 17 health districts. Here we aim at modeling the long term impact of SMC on *Plasmodium falciparum* clinical incidence and prevalence in the targeted population as well as in the general population. We used baseline and post intervention data on clinical and parasite rate detected by RDT and accounted for the selection of parasites with decreased drug sensitivity to calibrate the EMOD agent-based mechanistic model. Our 5 years simulations with a scenario of 80% SMC coverage were proven to substantially drop the prevalence of clinical malaria and parasite rate with a community based effect on *P. falciparum* prevalence in the general population.

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SPRAYING OF MALE MATING SWARMS AS A NOVEL VECTOR CONTROL INTERVENTION: INSIGHTS FROM A MATHEMATICAL MODEL

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Despite the success of long-lasting insecticide treated nets (LLINs) and indoor residual spraying (IRS) in controlling malaria transmission, challenges posed by elimination in residual transmission settings and waning effectiveness of current interventions prompt the investigation of novel vector control tools. Interventions that target hitherto unaddressed elements of vector biology may be of particular interest. Targeted insecticidal spraying of male mating swarms is a novel intervention that reduces the mating rate of female mosquitoes, thereby decreasing population density and biting rates. The only field trial of this intervention to date was undertaken in 2013 in Burkina Faso where it proved highly effective at reducing densities of *Anopheles gambiae*, one of the most dangerous African malaria vectors. Trial results showed that over a single month, mosquito densities were reduced by 80% in an ecological setting characterized by exceptionally high vector density. To understand the mechanisms through which spraying of male mating swarms impacted vector populations, a differential equation based model was developed and fitted to data. Parameters estimated through the model fitting procedure can give insight into mosquito mating dynamics in the natural setting as well as under an extreme external stressor. These results can give insight into how other interventions targeting mating dynamics may function. Results also allow an understanding of how female mating rate changes as males become scarce, indicating the importance of Allee effect driven population dynamics. The model-based analysis of these data suggest further research into targeting mosquito mating dynamics for vector control should be explored across a variety of ecological settings, both to better understand mosquito mating dynamics, as well as to evaluate the potential impact of swarm spraying across a variety of settings.

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DEVELOPING AN EARLY WARNING SYSTEM FOR MALARIA IN THE AMAZON: PROGRESS IN PERU

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Reported cases of malaria in the Peruvian Amazon have increased 5-fold since 2011, from 11,779 to 60,254 in 2015. Given the rate of detection up to April of this year, the number of cases are likely to exceed 70,000 in the region of Loreto. Responding to this epidemic has been challenging given the reduced financial support from the Peruvian government combined with large-scale environmental impacts that have affected Peru, including massive flooding in 2012 and the current "mega" El Nino. An

underappreciated driver of malaria transmission is the human-environment interface resulting in increased human contact with high-risk transmission environments. With support from NASA, a team of investigators from the US, Peru and Ecuador are developing a 2-component system that will improve detection of high-risk environments using large-scale prediction models, followed by application of focused agent-based models (ABMs) to predict local transmission dynamics and simulate potential intervention strategies. This presentation will provide an update of the system after one year of development. Data include: weekly case detection reports for 2000-2015 from all health posts in the region of Loreto in the northern Peruvian Amazon; satellite-derived estimates for meteorology, land cover, hydrology, and eco-region; and estimates of population density. The large-scale model identifies districts with high spatial and temporal clustering using polynomial distributed lag models. Initial formulations of these models have been presented previously. The small-scale ABMs, described in Pizzitutti et.al. 2015, have been improved to incorporate human mobility. This long-term project has both scientific and political (implementation) challenges that will be discussed.

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REQUIREMENTS FOR EFFECTIVE CRISPR-CAS9-BASED GENE DRIVE FOR THE CONTROL OF MALARIA AND OTHER VECTOR-BORNE DISEASES

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Recent demonstrations of CRISPR-Cas9-based gene drive by Gantz et al. and Hammond et al. highlight the huge potential of this technology for the control of mosquito-borne diseases such as dengue, Zika and malaria through genetic modification of their vectors. Two promising strategies have been proposed - one in which the gene drive system is used to spread a disease-refractory gene into the mosquito population, and another in which the gene drive system is used to spread a fitness load or male gender bias, thus suppressing the mosquito population. While encouraging as a proof-of-concept, these constructs are highly error-prone and induce large fitness costs, leading to certain failure in the wild. Using a population modeling framework, we determine minimal properties that these systems must have in order to be successfully implemented. Required improvements address the following issues: a) disruption of the CRISPR-Cas9 target site through non-homologous end-joining (NHEJ); b) fitness costs induced by the transgene and/or gene drive system; and c) evolution of pathogen resistance to the disease-refractory gene. We use our modeling framework to explore potential solutions to these issues. For instance: a) the use of second or third-generation CRISPR-Cas9 systems to enable sustained population replacement or suppression; and b) the use of CRISPR-Cas9 systems that prevent the accumulation of NHEJ-generated mutant alleles by cleaving a target sequence in an essential gene while also containing a recorded copy of the essential gene. Finally, we project the dynamics of the constructs of Gantz et al. and Hammond et al. beyond the experimental data, and explore tailored improvements. High homing rates were observed for both constructs, with over 90% transmission of the CRISPR-Cas9 system to offspring of heterozygous parents (c.f. 50% expected for purely Mendelian inheritance); however, high rates of NHEJ were also recorded, and females heterozygous for the construct of Hammond et al. had their fertility reduced by 90%. We recommend specific improvements for each system and thus suggest research priorities for the CRISPR-Cas9 gene drive field.

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MODELING THE USE OF A 20-HYDROXYECDYSONE STEROID AGONIST FOR MALARIA CONTROL

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Mathematical models have played an important role in designing malaria control policies by relating aspects of mosquito biology and behavior to malaria transmission. We developed a discrete-time compartmental model of the mosquito lifecycle to investigate the use of a novel compound for impregnated bed nets. This compound, a dibenzoylhydrazine, is a 20-hydroxyecdysone (20E) steroid hormone agonist with multiple effects on *Anopheles gambiae*, an important vector of the malaria parasite *Plasmodium falciparum*. The application of this 20E agonist to lab-reared mosquitoes reduced their mating success and egg development, shortened their lifespan, and blocked parasite development - effects that interfere with their ability to transmit malaria. We incorporated these varied effects into our model to predict the impact of this 20E agonist on the mosquito population and malaria transmission when applied via bed nets at varying levels of coverage. We find a non-linear relationship between bed net coverage and the mosquito population size, with a slightly increased population size at low coverage due to density-dependent larval mortality. However we predict, at all coverages, a shift in the mosquito age distribution toward younger mosquitoes, which are unable to transmit malaria, and reduced malaria prevalence in the human population, with the possibility of elimination at high coverage. These results show the potential of 20E agonists as new compounds for malaria control and highlight the utility of mathematical models when studying multiple aspects of mosquito biology that are simultaneously affected.

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WITHIN-HOST MATHEMATICAL MODELS OF MALARIA BUILT FROM MULTI-OMIC DATASETS

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The characterization of a malaria infection via a mathematical model that takes into account essential molecular interactions has been elusive. In the recent past, mathematicians trying to characterize the behavior of molecular and/or cellular quantities relied on simple diagrams (i.e. involving a reduced set of quantities) of processes described by biologists. Now instruments produce up to billions of measures in a single run from samples isolated from a particular biological domain (genomics, transcriptomics, metabolomics, proteomics, etc). The -omics revolution has changed the overall situation from data-poor to data-rich and thus has put many modelers in a situation of data overload. In this talk we address the integration of multiple -omic technologies to characterize the pathogenesis of malaria. Particularly, we will see how metabolic, transcriptional, lipidomic, and clinical data collected from a non-human primate infection with *Plasmodium* Cynomolgy can be used in unison to produce a mathematical model capable of differentiating between susceptible and resilient individuals. Such model is based on transport partial differential equations involving red blood cells, the immune system, and parameters that are functions of molecular quantities. This model makes use of a novel approach to cluster time series. The outcome of this model is a quantitative prediction of the course of a malaria infection based on known and anticipated molecular interactions that take place within a single individual. The lessons learned and methods developed during the course of this research are of ample applicability, thus the benefit of this aggregated knowledge expands beyond the field of malaria and outside the realm of research.

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ORDE WINGATE'S SUICIDE ATTEMPT, CAIRO, 1942: A CASE STUDY IN ACUTE ATABRINE PSYCHOSIS

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One of the best known field commanders of the Second World War was Orde Wingate. His daring guerilla tactics in British-mandated Palestine, Ethiopia, and Burma have become legendary among military historians. Indeed, Wingate's military career in the field has rightly attracted the bulk of attention of biographers and military historians. Such well-deserved attention to Wingate's military career, however, has tended to divert attention from a 1942 suicide attempt. Suffering from malaria—possibly cerebral malaria--Wingate received atabrine therapy from his physician. According to his biographers, he abruptly increased its recommended doses. In a botched suicide attempt that followed, Wingate thrust a knife into his neck. This proposal will examine the probable cause of Wingate's suicide attempt. Was it, as some observers suggest, the severity of Wingate's malarial condition? Was it the high dose of atabrine with which Wingate recklessly self-medicated that caused his acute psychosis. Or was it, in fact, a combination of the two, exacerbated by an undiagnosed psychiatric condition? The authors conclude that pre-existing psychiatric issues (which the overdose of atabrine exacerbated)—not cerebral malaria—precipitated his 1942 suicide attempt.

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JOINT EFFORTS, A KEY TO SUCCESS FOR THE MALARIA IN PREGNANCY PROGRAM IN LUANDA, ANGOLA

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Angola, in response to WHO's 2012 updated guidance on Intermittent Preventive Treatment in pregnancy (IPTp), revised its national malaria protocol to better address the fact that 25% of maternal mortality is caused by the disease. The new protocol was a collaborative effort of a national technical working group assisting the National Malaria Control Program (NMCP) including the National Reproductive Health Program, the national AIDS Institute, WHO, UNICEF, UNFPA and implementing partners of the U.S. Presidents Malaria Initiative (PMI). The updated Prevention and Treatment Manual for Malaria in Pregnancy, based on the revised protocol, was approved in 2014, and efforts continued with reviewing and updating training modules, job aids and monitoring tools that would reflect the additional doses of IPTp. The Ministry of Health, with support from partners, then disseminated these materials in the provinces and municipalities where they worked. USAID's ForçaSaúde program, with support from PMI, worked with the Provincial Health Directorate of Luanda to build capacity of 297 health professionals to implement the new guidance in 78 health facilities of four municipalities, Belas, Cazenga, Cacuaco and Viana, with a combined population of 4.3 million. Comparing the IPTp data from the four municipalities between 2014 and 2015, one can see that the new guidance has started to take effect. In both years approximately 70,000 pregnant women received the first dose or around 60% of women registering for antenatal care (ANC). For the new third dose there was an increase of 85% (from 12,490 women to 23,046), and receipt of the fourth dose rose by 164% (3,345 to 8,839). Two major challenges remain: increasing ANC registration and addressing missed opportunities to provide ANC doses for those who do attend including ensuring regular supplies of sulfadoxine-pyrimethamine for IPTp. Future progress requires continued inter-departmental collaboration among NMCP, Reproductive Health and the AIDs Institute, on-the-job training, enhanced statistical capacity, and supervision.

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STUDY ON PATIENT ADHERENCE TO CHLOROQUINE AND PRIMAQUINE TREATMENT FOR *PLASMODIUM VIVAX* MALARIA IN MANAUS, STATE OF AMAZONAS

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Compliance to treatment is one of the most important factors to ensure radical cure, avoid relapses, prevent the emergence of parasite resistance to anti-malarial drugs and decrease transmission. Brazil has been using the "short" treatment scheme for *Plasmodium vivax* malaria - 3 days chloroquine +7 days primaquine - for many years trying to ensure patient`s adherence. Currently the country also use, when adherence could be secured, the "long" scheme with 14 days primaquine (or primaquine adjusted by weight). This study was conducted in Manaus, Amazonas to assess patient adherence to the standard *P. vivax* malaria scheme with 3 days chloroquine+7 days primaquine in the health centers' routine care; and identifying factors that determine low treatment adherence and adverse events associated with the treatment of *P. vivax* malaria. It was a transversal study conducted with patients seen in 11 health centers and designed to quantify adherence by conducting home interviews and verifying how many pills were remaining one day after the last treatment day. Sample size was determined by using an expected 15% proportion of low adherence, a 95% confidence range, and a 5% level of significance. 165 patients were interviewed, 98 were male and 67 female, and 100 were the patients themselves and 65 their guardians.There were a total of 31 patients who failed to adhere, representing 18.8% of the total, of which 6.1% had blisters with pills and 12.72% reported non-adherence. The study demonstrated problems in adhering to primaquina related to various factors that vary from the instructions given by the dispensers but not really understood by patients to the presentation of the medications. The value found for non-adherence/probable non-adherence may still be understated due to the possible introduction of selection biases: patients who are available for a visit and interview may cooperate more, for example. The result of this study reinforces an important problem with the *P. vivax* scheme that need to be addressed. In view of the malaria elimination interest it is important to show problems that need to be solved in order to reach the elimination goal.

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PRE-SERVICE TRAINING INSTITUTIONS: AN IMPORTANT CONTRIBUTOR TO SCALE UP OF HIGH QUALITY MALARIA CASE MANAGEMENT SERVICES IN MALAWI

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Pre-service training institutions are an important, but often neglected, component of improving the quality of malaria case management. In 2007, Malawi updated its national guidelines on first line malaria treatment (from sulfadoxine-pyrimethamine to the artemisinin-based combination therapy (ACT) artemether-lumefantrine). By 2012, the Ministry of Health began reviewing the corresponding national training curriculum to orient health providers to the new malaria case management treatment guidelines. To support rollout, MalariaCare partnered with the National Malaria Control Program to train 3,035 health care providers (across 14 districts) on the updated national case management guidelines. Between 2007 and 2014, limited efforts were made to target pre-service training institutions as well. To enhance timely scale up of high quality case management services, MalariaCare trained 22 lecturers from eight training institutions, representing half of all training institutions in Malawi. Participants' knowledge and competencies related to the updated malaria case management guidelines were evaluated through pretests and posttests. Key findings include that average scores increased

from thirty eight percent (38%) at pretest to seventy three percent (73%) at posttest - indicating that baseline knowledge of the updated malaria case management guidelines was low among this group. Next steps for ensuring that new graduates continue to be well prepared will also be shared, including partnering with training institutions and Malawi's National Malaria Control Program to orient final year students to Malawi's updated malaria case management guidelines and support for incorporating the updated national guidelines into pre-service training curricula. In addition to practicing health providers, training institutions should be engaged to ensure that new health providers entering the workforce are up to date on changes to national guidelines, allowing them to contribute to the scale up of high quality malaria case management services.

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IMPROVING QUALITY OF MALARIA CASE MANAGEMENT IN MALAWI THROUGH TARGETED CLINICAL MENTORING

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The Malawi Ministry of Health estimates that malaria accounts for 34% of all outpatient visits and 40% of all hospital admissions, with approximately 5 million cases treated each year. MalariaCare seeks to test promising new methods for more rapidly improving the quality of malaria diagnosis and treatment. In Malawi, the project is working closely with the National Malaria Control Program, Malaria Alert Center and Queen Elizabeth's Hospital to enhance routine outreach training and supportive supervision (OTSS) efforts by supporting a core group of clinical experts to provide intensive clinical mentoring to health facilities that are not meeting the project's minimum standards for quality case management. Intensive mentoring is used to target areas of weakness using problem-solving approaches and experiences that have worked in similar settings to address challenges and improve performance. Intensive mentoring is a collaborative approach engaging the commitment of both mentor and mentee. MalariaCare's hypothesis is that intensive mentoring will improve performance in a sustainable way on gaps identified during OTSS. Given the lack of literature on the contributions of clinical mentoring towards improved quality of malaria case management, MalariaCare will share findings regarding the use of mentoring toward this purpose in select health facilities. A core group of clinical mentors has been trained to provide intensive clinical mentoring in selected high-volume low-performing health facilities, following supervision rounds and through remote support by phone and email. Data will be collected using a standardized malaria case management evaluation tool developed by MalariaCare. A brief competency-based pre-post assessment tool will be used to assess provider competencies at the beginning and end of the mentoring process. The primary outcomes, however, will be changes in performance on case management indicators after mentoring and over time compared with a baseline. Results and lessons learned are expected to generate evidence on the potential role of clinical mentorship for improving the quality of malaria case management.

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CLINICAL, DEMOGRAPHIC AND LABORATORY DATA AND METADATA COLLECTION FOR HUMAN MALARIA BLOOD SAMPLES COLLECTED FROM INDIVIDUALS LIVING IN DIVERSE EPIDEMIOLOGICAL SETTINGS

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Malaria is a leading cause of disease and death in many developing countries and is a severe public health problem worldwide. Independent research centers are active in many malaria endemic countries in Asia, Africa, and South America. To enhance the effectiveness of global malaria research and help accelerate progress, collaboration and data sharing between and among national and international research centers and other facilities is critical. Such collaboration is possible when easy to follow standards for data and metadata collection and dissemination are available and widely adopted. The NIH/NIAID-supported Malaria Host Pathogen Interaction Center, MaHPIC, consortium involves international collaborators from Brazil, Thailand, Colombia, Malaysia and Nigeria, with more coming onboard. We have collected clinical, demographic and laboratory data and metadata from diverse research collaborations from around the world, and developed easy to use data entry templates that are compatible with the data standards developed by the International Centers for Excellence in Malaria Research (ICEMRs) and the NIH/NIAID Clinical Data Working Group. The challenges faced with respect to mapping terms to the currently available and accepted ontologies will be discussed and solutions provided.

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USING OUTREACH TRAINING AND SUPPORTIVE SUPERVISION (OTSS) RESULTS TO MONITOR ADHERENCE TO REVISED MALARIA TREATMENT GUIDELINES IN THE EASTERN REGION OF GHANA

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Following WHO's 2010 recommendation, Ghana revised its national malaria case management guidelines in 2014 to include laboratory confirmation for all suspected malaria cases before treatment with ACT. Training was conducted for public health facility workers on the new guidelines, but training numbers were inadequate. MalariaCare supports the National Malaria Control Program to conduct regular outreach training and supportive supervision (OTSS) visits to health facilities as part a strategy to improve adherence to the new guidelines and overall provision of febrile case management in five supported regions. The first round of OTSS following the training on updated guidelines resulted in a total of 426 health facilities receiving supervision visits. These visits included observations, practical demonstrations, interviews and patient record reviews to identify weaknesses, encourage problem solving and strengthen performance through on-the-job training and mentoring. OTSS data from this round shows that 82.9% of staff interviewed had participated in case management training, and that the overall score for clinical fever evaluations observed was 92.7%. The clinical fever evaluation score is a composite of the following: in 92.2% of observations, clinicians appropriately classified fever and 95.5% requested a malaria test to confirm the clinical diagnosis. Following a diagnostic test, 81.8% adhered to a negative test result and 87.6% prescribed according to guidelines as observed by supervisors during patient encounters. In a review of facility clinical records, adherence to a negative test result was higher at 92.7% of the sampled records. Comparison with the two previous rounds of

OTSS, which occurred before training on the updated guidelines, indicated improvement in performance of all four clinical fever evaluation indicators to above 80%, and an improvement in adherence to negative test results from 57.2% to 81.2%.

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LESSONS LEARNED: MALARIA CASE MANAGEMENT TRAINING IN MADAGASCAR

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To help scale up high-quality diagnosis and case management services for malaria and other febrile illnesses, MalariaCare is supporting Madagascar's National Malaria Control Program (NMCP) to conduct clinical case management training in the Menabe region, with training sites in Morondava, Belo, Miandrivazo and Mahabo. Training materials were aligned with NMCP guidelines, with emphasis on orienting participants to Madagascar's updated policy around first-line treatment (three days of ACT), use of artesunate injection for severe malaria, as well as clinical and diagnostic practices. Participants were assessed using pretests and posttests. Of the 55 participants, 71% were nurses and 29% were physicians. Overall, 85% of participants stated that the training met their expectations. In addition, 87% of participants in Morondava improved their knowledge and skills after training - with an average increase of 16 percentage points between pretest and posttest. Similarly, 80% of participants in Belo improved their scores by an average of 17 percentage points, 94% of participants in Miandrivazo increased their scores by an average of 18 percentage points, and 93% of the participants in Mahabo increased their scores by an average of 18 percentage points. Average pre and posttest scores (out of 30) were 20 and 25 for Morondava; 18 and 23 for Belo; 19 and 25 for Miandrivazo, and 20 and 25 for Mahabo. Participants will use the knowledge gained from this training to improve the quality of diagnostic and treatment in their communities, aimed at reducing malaria related morbidity and mortality rate in remote areas of Madagascar.

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ASSESSING THE PERFORMANCE OF AN INTEGRATED DISEASE SURVEILLANCE AND RESPONSE SYSTEM IN THE CONTEXT OF VARYING MALARIA TRANSMISSION: A CASE STUDY FROM MADAGASCAR

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The Madagascar's ministry of health (MOH) is building an integrated health information system to streamline reporting at all levels. One component of this is an integrated disease surveillance and response system (IDSR) for notifiable diseases. Madagascar's entire population is at risk of malaria with epidemic prone areas. As malaria transmission decreases and Madagascar considers pre-elimination, a specific surveillance system is also required. In October 2015, the MOH conducted an assessment of the IDSR in 93 randomly selected health facilities (HF), 218 community workers, and 19 sentinel sites. Results showed that 70% of HF reported accurate data, 62% of expected reports sent to district, 50% of expected reports received on time. IDSR guidelines were found in 20% of HF, 37% of HF have the official list of diseases under surveillance, and 54% have the weekly reporting form. Only 50% of IDSR staff at the district level, 57% at the region level were trained in surveillance. Supervision was irregular with 50% of HF supervised in the past six months. Only 52% of districts and 3/10 regions have an epidemic management committee. Data use was limited, only 40% of districts could investigate all epidemic alerts in the past 12 months. These findings suggest the need to develop and implement a comprehensive IDSR-strengthening strategy including data quality assurance procedures, use of new technologies, staff capacity building, coordination among partners, and use of data to respond to disease control in Madagascar.

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MONITORING AND EVALUATION WORKSHOPS: AN APPROACH TO IMPROVE MALARIA INFORMATION SYSTEMS AND DATA USE TO BETTER INFORM PROGRAM IMPLEMENTATION

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Malaria control interventions in most endemic countries have intensified in recent years and there is a need for robust monitoring and evaluation (M&E) systems to measure progress and achievements. Providing program and M&E officers with appropriate skills is a way to strengthen malaria's M&E systems and enhance information use for programs' implementation. From 2010 to 2015, capacity strengthening efforts included organizing regional in-person workshops for M&E of malaria programs for Anglophone and Francophone countries in sub-Sahara Africa in collaboration with academic institutions from Ghana and Burkina Faso. Open-sourced online courses were also available in English. A post-workshop assessment was conducted after five years to assess the effects of these regional workshops and identify gaps in capacity. The regional workshops trained two hundred and eighteen participants from 28 countries from 2010 to 2015. Trained participants were from ministries of health, national malaria control programs, non-governmental organizations, and development partners. The average score (%) for participants' knowledge tests increased from pretest to posttest for the Anglophone workshops (2011: 59 vs. 76, 2012: 41 vs. 63, 2013: 51 vs. 73; 2014: 50 vs. 74, 2015: 52 vs. 69). Similarly, Francophone workshop posttest scores increased, but were lower than Anglophone due to higher scores at pretest. (2011: 70 vs 76, 2012: 74 vs 79, 2013: 61 vs 68; 2014: 64 vs 75, 2015: 70 vs 79). Results of the post-workshop assessment revealed that participants retained practical M&E knowledge and skills for malaria programs, but there is a need for a module on malaria surveillance adapted to the pre-elimination context. The workshops were successful because of the curriculum content, the facilitation quality, and the engagement of partner institutions with training expertise. Results from the post-workshop assessment will guide the curriculum's development and restructuring for the next phase of workshops. Country-specific malaria M&E capacity needs assessments may also inform this process as countries reduce malaria burden.

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MAPPING MALARIA RESEARCH FOCUS, CAPACITY AND INTERNATIONAL COLLABORATION IN NIGERIA

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Nigeria has the highest burden of malaria globally with the entire population (est. 182 million) at risk of infection and disease. Nigeria is scaling up its efforts to control and eliminate malaria, which requires a wide range of country-specific technical support and scientific expertise for mapping, monitoring and evaluating, operational research and documenting programmatic impact and success. To better understand the distribution of skills and institutional capabilities that may provide programmatic support within Nigeria, this study aims to identify and map malaria research focus, areas of scientific capacity and international collaborative links by analyzing data from published scientific articles. Using electronic searches in online bibliographic archives, a systematic collation of articles from the past 5 years with at least one Nigerian-based author is being conducted. From this, a comprehensive geo-referenced database is being developed by categorizing key information including authors, institutions, location (i.e. state, zone, place), research type (i.e. molecular, parasitological, immunological, epidemiological and/or entomological), journal, journal impact factor, number and country of international collaborators and main sources of funding. To date, more than 400 articles on malaria research in Nigeria have been identified and being entered into the database. Maps will be presented on the number of articles, institutional type, and research focus with key collaborative networks and funding sources highlighted. The study will provide a useful resource for the national malaria programme as well as national and international scientists. It will highlight the current technical and scientific potential that could be maximized for programmatic purposes, and also provides a model approach that can be applied in other endemic counties.

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THE INCUBATION PERIOD OF MALARIA AMONGST TRAVELERS RETURNING TO THE UK

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The introduction of imported cases of malaria to non-endemic countries remains an on-going concern for healthcare professionals. The United Kingdom (UK) alone continues to receive and treat ~ 1,500 cases of imported malaria each year. This number remained roughly constant between 2003 - 2013. Accurate quantification of the mean and variance of this key epidemiological parameter is essential to inform public health policy and specify case definitions. To reduce the risk of severe malaria and death associated with infection, rapid diagnosis has been demonstrated to be key for prevention. However, to date, few studies have estimated the incubation period of malaria collectively from a large number of individuals. Current UK guidelines available to the public suggest that the incubation period of malaria is approximately 7 -18 days. We use data on clinically confirmed cases of *Plasmodium falciparum* and *Plasmodium vivax* imported to the UK between 1991 - 2006. Amongst cases of in travellers arriving in the UK, onset of symptoms occurred within a mean of 19 days after arrival for *P. falciparum*. For *P. vivax* the observed incubation period distribution was bimodal with the first peak of infections reported with a mean of 22 days, the data showed the classic second peak around 265 days post infection for *P. vivax*. For a subset of cases, the use of anti-malarials and less severe malaria infection lengthened the mean incubation period of *P. falciparum* from 13.4 days, by 4 days and 5.1 days respectively in a multivariate regression model. For *P. vivax* our findings demonstrated that women had shorter incubation periods. Our findings highlight that while for some cases the incubation period falls within the current recommended guidelines, on average the reported estimated incubation period duration was longer, particularly for *P. vivax*.

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THE USE OF RESPONDENT-DRIVEN SAMPLING TO ASSESS MALARIA KNOWLEDGE, TREATMENT-SEEKING BEHAVIORS AND PREVENTIVE PRACTICES AMONG MOBILE AND MIGRANT POPULATIONS IN AN ARTEMISININ RESISTANCE SETTING IN WESTERN CAMBODIA

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The movement of populations within and between malaria-endemic regions is a major contributor to the spread of artemisinin resistance along the Cambodian borders. Mobile and migrant populations are poorly connected to public health and surveillance systems. Their high mobility makes them difficult to survey and reach with health interventions. The objective of this study was to determine knowledge of malaria, treatment-seeking behaviours and preventive practices among migrant/mobile workers in Western Cambodia. A structured survey of mobile and migrant populations was implemented in two provinces of Western Cambodia along the Thai-Cambodian border using a Respondent Driven Sampling (RDS) approach. Results: There were 764 participants in Pailin and 737 in Veal Veng. Most respondents (93.7% in Pailin and 96.1% in Veal Veng) received health messages, predominantly on malaria. Knowledge of malaria transmission, prevention, and symptoms was found to be very high (94.4% in Pailin and 98.2% in Veal Veng); however, other beliefs persist, predominantly in Veal Veng (24.4% listed a dirty or unsanitary environment and 57.6% listed contaminated food or drink in Veal Veng). Knowledge of using a mosquito net was high (95.5% in Pailin and 99.1% in Veal Veng), however specific knowledge of the use of an insecticide treated bed net (ITN) for prevention was relatively low. Stated ownership of an ITN or treated hammock net was low (25.3% in Pailin and 53.2% in Veal Veng). Of those that took an antimalarial, greater than 60% used artemisisnin-based combination therapy (ACT) or atovaquone-proguanil. In conclusion, RDS sampling methodologies were used to effectively statistically robust data to evaluate malaria risk and to assess protective behaviours of this difficult-to-survey population. There is a need for sustained public health efforts to reach these populations within an elimination context. These findings are critical for informing strategies to more effectively deliver health services and promote behaviour change among a population at high risk for contracting and spreading artemisinin-resistant malaria.

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LACK OF MORTALITY IN CHILDREN WITH SICKLE CELL DISEASE AND SEVERE MALARIAL ANEMIA WHO RECEIVE TIMELY BLOOD TRANSFUSION

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Hemoglobin AS (sickle cell trait) has a strong protective effect against malaria, particularly severe malaria, but studies with small numbers of children with HbSS (sickle cell anemia, SCA) and severe malaria have suggested that children with SCA who develop severe malaria have a high mortality rate. To better characterize hematologic parameters and risk of mortality in children with SCA and severe malaria, we compared these factors according to HbS genotype (AA, AS, SS) in a cohort of children 18 months – 12 years of age with severe malarial anemia (SMA, n= 184), cerebral malaria (CM, n= 213) or healthy community controls (CC, n= 197). All children with SMA received blood transfusion. HbAS (41, 6.9%) was more frequent in CC (37, 18.8%) than in SMA (2, 1.1%) or CM (2, 0.9%, *P*<0.001), confirming the protective effect of HbAS against severe malaria. HbSS (18, 3.0%) was more frequent in SMA (17, 9.2%) than CM (1, 0.5%, P<0.001). Among children with SMA, children with HbSS and HbAA had similar hemoglobin levels, but children with HbSS had a higher WBC count and lower parasite density (both *P*<0.001). There was no mortality in children with SMA, even in those with HbSS. Our findings show that SCA is a major risk factor for SMA in this population, but SCA is not associated with increased mortality in SMA if timely transfusion is available.

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AGE- AND PREVALENCE-RELATED MALARIA INFECTION RISK AND TREATMENT BEHAVIOR: EVIDENCE FROM A HOUSEHOLD SURVEY IN UGANDA

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In Sub-Saharan Africa, both under-treatment and over-treatment of malaria are common since illnesses are often diagnosed and treated on the basis of symptoms. Using data on fever treatment and malaria rapid diagnostic testing from 2,285 households in 92 villages in Uganda, we find that although households treated 40% of clinical malaria episodes with artemisinin-based combination therapies (ACTs)—the first-line, most effective, class of drugs for the disease—households also treated the same proportion of non-malarial febrile illnesses (Unadjusted Difference: -0.036, 95% CI: [-0.111 0.039], p=0.345). We show that both the age of the patient and the village prevalence rate are strongly associated with the probability that a febrile patient is infected with malaria: each additional age year is associated with a 0.6 percentage point decline in the probability of testing positive for malaria (95% CI: [-0.008 -0.005], P<0.001), and a one standard deviation increase in the village malaria prevalence rate is associated with a 13 percentage point increase in the probability that a febrile patient is infected with malaria (95% CI: [0.105 0.154], P<0.001). However, ACT treatment rates for febrile illnesses are not significantly associated with either age (Unadjusted Coefficient: -0.001, 95% CI: [-0.003 0.001], P=0.180) or with standardized village malaria prevalence rates (Unadjusted Coefficient: -0.032, 95% CI: [-0.074 0.009], P=0.121). We also present some evidence that whether a caregiver believes a febrile illness is malaria is unrelated to the febrile patient's age and to the local prevalence rate, suggesting that information gaps on malaria risk may play a large role in the under-treatment of malaria.

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RELATIONSHIP BETWEEN THE PREVALENCE OF PARASITEMIA IN PREGNANT WOMEN AND CHILDREN: BIOKO ISLAND MALARIA INDICATOR SURVEY 2008-2015

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Pregnant women have been one of the main targets in the efforts to control malaria and in some settings they are routinely screened and treated or provided anti malaria prophylaxes and also provided ITNs. Studies have indicated that they are more likely to have detectable malaria due to higher parasite densities. Prevalence in children (2-14 years) is one of the main standard measures used to monitor the outcomes and impacts of malaria control programs in moderate to high transmission areas. Pregnant women attend ANC and blood samples are routinely taken and could be used to screen for parasitemia, allowing for an insight into the monthly variation of the incidence of malaria without need for extra sampling. Knowing the relationship of the prevalence of malaria in children and pregnant women could have far reaching implication the various control interventions in terms of timeliness of data and the implementation of the routine MIS. Using 8 years data of the annual MIS Bioko islands of Equatorial Guinea from 2008-2015, the yearly prevalence of parasitemia in children and pregnant women was calculated and Pearson's product-moment correlation was run to assess the relationship. Prevalence among children is relatively higher, there exist similar trends in the two groups; There was a very strong positive correlation between prevalence of the two groups $r(6) = 0.909$, $p < .002$. In conclusion, ANC attendees can be used as a sentinel group to monitor malaria prevalence because they are readily available and show similar trends with the children whose malaria prevalence is a standard measure to estimate malaria endemicity. More importantly, there will be timely and seasonal data for decision making.

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ONE YEAR STABILITY ANALYSES OF PFS25-EPA AND PFS230-EPA CONJUGATES ADJUVANTED WITH ALHYDROGEL®

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Plasmodium falciparum Pfs25 and Pfs230 proteins are important malaria transmission-blocking vaccine candidates. Pfs25 is expressed as a surface protein during zygote and ookinete stages in infected mosquito, while Pfs230 is expressed in gametocytes in the human host and on the surface of gametes in the mosquito host. To increase immunogenicity, the recombinant Pfs25 and domain 1 of Pfs230 (referred to as Pfs25M and Pfs230D1M) were conjugated to the recombinant, nontoxic *Pseudomonas aeruginosa* ExoProtein A (rEPA) in conformance with current good manufacturing practices (cGMP). The resultant clinical grade conjugates (Pfs25M-EPA and Pfs230D1M-EPA) were formulated on Alhydrogel at 78 µg/mL for Pfs25M and 50 µg/mL for Pfs230D1M. To ensure the vaccines are in compliance with the regulatory specifications during the phase 1 human clinical trial period, annual evaluation of these vaccines was performed. The one year stability analyses on the clinical lots included appearance, endotoxin content, sterility, strength (protein content tested by *o*-Phthaldialdehyde assay), identity (SDS-PAGE and Western blot after antigen extraction from Alhydrogel), integrity (pH, percent protein bound to Alhydrogel, SDS-PAGE, Intrinsic Fluorescence CD, Direct Alhydrogel Formulation Immunoassay), and efficacy (the mouse potency assay). Our results showed that the Drug Products Pfs25M-EPA and Pfs230D1M-EPA formulated on Alhydrogel are biochemically, biophysically, and biologically stable after storage for one year at 4ºC. Thus, we conclude that sufficient stability of these candidates supports further studies in human clinical trials.

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LONG TERM IMMUNE RESPONSES INDUCED AGAINST *PLASMODIUM VIVAX* CSP AND MSP-1 CHIMERIC VACCINE CANDIDATES DELIVERED BY NOVEL ADENOVIRAL VECTORS

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Protection against malaria based on pre-erythrocytic and erythrocytic antigens depends on IFN-γ production by CD4 and CD8 T cells. Adenoviral vectors have been used to induce robust cellular immune responses in recent malaria vaccine clinical trials and reports indicate that adenoviruses are capable of long periods of transgene expression, a desirable for malaria vaccines since immune responses lasting several transmission cycles are necessary to exert a substantial effect on disease incidence. To test the durability of the immune responses induced by adenoviral vectors encoding a multistage *Plasmodium vivax* chimeric protein vaccine including promiscuous T cell and B cell epitopes from the circumsporozoite protein (CSP) and merozoite surface protein-1 (MSP-1), we primed BALB/c mice with one of two different recombinant adenoviral vectors and assessed their immune responses and ability to induce memory T cells by boosting the mice one year post priming. We found that mice immunized with the recombinant proteins or one of the two protein-encoding adenoviral vectors - Simian Adenovirus 36 (SAd36) or a recombinant human adenovirus 5 modified to contain the fiber-knob region of adenovirus serotype 3 (Ad5/3) - displayed no significant decrease in antibody titers against the CSP and MSP-1 protein chimeras when antibody titers were compared between day 20 and 1 year post priming. We subsequently boosted mice with the heterologous adenoviral vector 1 year post priming, followed by two protein boosts 20 and 40 days later. We found that in comparison to mice receiving only protein immunizations, both heterologous adenoviral regimens induced CD4 and CD8 T cells capable of producing higher levels of IFN-γ, IL-2, and TNF-α upon *ex vivo* stimulation with the MSP-1 or CSP chimeric recombinant proteins or peptide pools representing T or B cell epitopes derived from these chimeric proteins. Taken together, the data demonstrate that our novel multistage malaria vaccine is able to induce long lasting humoral and cellular immune responses able to recognize two different *Plasmodium* antigens when delivered by a regimen that includes adenoviral vectors.

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A HYBRID *PLASMODIUM VIVAX* BLOOD STAGE-TRANSMISSION BLOCKING VACCINE CANDIDATE ELICITS ROBUST CELLULAR IMMUNE RESPONSES AND LONG-LIVED FUNCTIONAL ANTIBODIES

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Malaria control efforts focused on the use of vector based interventions have resulted in a significant reduction of *Plasmodium falciparum* cases. Unfortunately, the unique biological features of *P. vivax* make vector control interventions ineffective against this parasite. Transmission Blocking Vaccines (TBVs) are considered the best alternative for achieving malaria control as antibodies induced by TBVs taken up by the mosquito during the blood meal interrupt parasite development. However, a major concern for post-fertilization TBVs is a short lived antibody response as the human host is not exposed to these mosquito stage antigens, needed to induce a natural boosting effect. A transmission blocking formulation could also result in low compliance since it would not be protective against the disease. To address these problems, we designed a multistage chimeric vaccine denominated *P. vivax* erythrocytic stage-transmission blocking chimera (PvES-TBC). This vaccine candidate includes 5 promiscuous T helper cell epitopes derived from the erythrocytic antigen MSP-1 which were genetically linked to the MSP-1 19kDa fragment and to the transmission blocking antigen Pvs25. PvES-TBC was tested in comparative experiments with Pvs25 in mice. PvES-TBC induced a strong cellular and humoral immunity able to recognize both MSP-1 and Pvs25. Additionally, when compared to Pvs25, PvES-TBC elicited a more robust antibody response as determined by a significantly higher avidity and a bias toward IgG2a production. This response was determined to last for a period longer than 2 years after the final boosting in mice immunized with PvES-TBC, an effect not observed in the Pvs25 group. The long lasting response was related to a significantly higher proportion of long lived plasma cells in mice immunized with PvES-TBC. When the transmission blocking ability of antibodies was tested by membrane feeding assays using wild *P. vivax* isolates, the antibodies produced in response to PvES-TBC were able to block 90% of mosquito infections. These characteristics make PvES-TBC a promising transmission blocking vaccine candidate that warrants clinical development.

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SAFETY AND IMMUNOGENICITY OF THE NOVEL *PLASMODIUM FALCIPARUM* BLOOD-STAGE VACCINE CHAD63-MVA RH5 IN A PHASE IA CLINICAL TRIAL

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The development of an effective malaria vaccine remains a vital goal if eradication is likely to be achieved. Candidate antigens for blood-stage *Plasmodium falciparum* vaccines have been hampered with issues of polymorphism and the need to induce extremely high antibody concentrations for these vaccines to be effective. The reticulocyte-binding protein homologue 5 (RH5) is the most promising blood-stage *P. falciparum* candidate antigen to date. It is essential for parasite survival and erythrocyte invasion and, unlike other blood-stage antigens (e.g. AMA1 and MSP1) RH5 does not appear to come under significant immune pressure in endemic settings, and there is limited polymorphism. Vaccination with an RH5-based vaccine confers protection against malaria challenge in non-human primates and the antibodies induced by vaccination are able to cross-inhibit *in vitro* all *P. falciparum* isolates tested to date. We report on a first-in-human Phase Ia clinical trial (NCT02181088) of an RH5-based vaccine delivered using the viral vectors chimpanzee adenovirus serotype 63 (ChAd63) and modified vaccinia virus Ankara (MVA) in a heterologous prime-boost strategy. The trial was conducted in Oxford and Southampton, United Kingdom and recruited twenty-four healthy, malaria-naïve volunteers aged 18-50. The first four volunteers (Group 1) received a lead-in dose (5×10^9 viral particles, vp) of the ChAd63 RH5 vaccine alone before dose escalation to the full dose (5×10^{10} vp) in Group 2. The first four volunteers in Group 2 received ChAd63 alone, and the final sixteen were boosted with MVA RH5 eight weeks after ChAd63 RH5 at doses of $1 – 2 \times 10^8$ plaque-forming units, pfu. Data on all adverse events were recorded for 28 days after each vaccination and serious adverse event (SAE) data were collected for the duration of the study. This is the first RH5-based vaccine to be tested in humans. The vaccines were well tolerated, with no safety concerns and there were no SAEs. T cell and serum antibody responses were assessed by *ex-vivo* interferon-γ ELISpot, ELISA and *in vitro* growth inhibition activity (GIA) assays. The vaccines were immunogenic and these data will be presented.

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ENHANCED PROTECTIVE EFFICACY OF A *PLASMODIUM FALCIPARUM* MALARIA VACCINE USING A HETEROLOGOUS PRIME-BOOST IMMUNIZATION WITH A BACULOVIRAL VACCINE AND CHAD63 EXPRESSING PFCSP AGAINST CHALLENGE WITH TRANSGENIC *P. BERGHEI* SPOROZOITES

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We have recently developed a new vaccine platform system based on the baculovirus *Autographa californica* nucleopolyhedrosis virus (AcNPV) called the baculovirus dual-expression system (BDES). BDES is capable of displaying an antigen on the viral envelope by the use of a baculovirus-derived polyhedrin promoter and expressing it upon transduction of mammalian cells by cytomegalovirus (CMV) promoter and so can function as both a vaccine component and a DNA vaccine, respectively. In our study, a heterologous prime-boost immunization regime using the newly-developed BDES vaccine and the clinically relevant recombinant chimpanzee adenovirus 63 (ChAd63) expressing the *Plasmodium falciparum* CSP (PfCSP) transgene was assessed for its protective efficacy and immunogenicity in a mouse model, using a rodent malaria *P. berghei* chimeric parasite expressing PfCSP as *in vivo* challenge model. We performed a series of animal experiments to evaluate protective efficacy of the heterologous immunization regime. BALB/c mice were primed with ChAd63 and boosted with BDES, and then challenged by intravenous injection of 1,000 *P. berghei* chimeric sporozoites. ChAd63-BDES immunization elicited higher protection than BDES alone with statistical difference. Assessment of immunological analysis is ongoing to find the relationship between immunogenicity and protective efficacy. These findings serve to inform us new insight to develop a new malaria vaccine using the ChAd63-BDES platform.

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CRYOPRESERVATION RELATED LOSS OF ANTIGEN SPECIFIC IFNγ PRODUCING CD4+ T-CELLS: LESSONS FROM A MALARIA VACCINE TRIAL SUBSTUDY

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Ex vivo functional immunoassays such as ELISpot and intracellular cytokine staining (ICS) by flow cytometry are crucial tools in vaccine development both in the identification of novel immunogenic targets and in the immunological assessment of samples from clinical trials. Cryopreservation and consequent thawing of PBMCs via validated processes has become a mainstay of clinical trials due to processing restrictions inherent in the disparate location and capacity of trial centres and also in the logistical and financial requirement to batch process samples from multiple study timepoints. Using ELISpot and ICS assays to assess antigen specific immunogenicity in blood samples taken from subjects enrolled in a phase II malaria heterologous prime-boost vaccine trial, this study has shown that the freeze thaw process can result in a 3-5 fold reduction of malaria antigen specific IFNγ producing CD3+CD4+ effector populations from PBMC samples taken post vaccination. We have also demonstrated that it is likely that peptide responsive CD3+CD8+ T-cells are relatively unaffected as well as more persistent CD3+CD4+ populations that do not produce IFNγ. These findings contribute to a growing body of data that could be consolidated and synthesised as guidelines for clinical trials with the aim of improving the analysis of vaccine candidates.

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RTS,S MALARIA VACCINE EFFICACY DOES NOT VARY WITH SEASONAL PRECIPITATION: RESULTS FROM LILONGWE, MALAWI

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Phase 3 trials of the efficacy and safety of the candidate malaria vaccine RTS,S are complete, but site-specific data are limited. This study assesses the interaction of precipitation and vaccine efficacy and corresponding estimates of clinical malaria episodes averted in a seasonal-transmission region of sub-Saharan Africa. We followed children (5-17 months of age) and infants (6-12 weeks of age) who were randomly assigned to a vaccine group, vaccine with booster group, or control group. Primary efficacy was defined as development of clinical malaria (fever ≥37.5°C and *P. falciparum* parasitemia >5,000 per microliter). Precipitation data were obtained from the Chitedze Agricultural Center in Lilongwe. Cox proportional hazards models were used to assess time until first malaria case. Effect modification was assessed by including interaction terms for vaccination status and precipitation. Vaccine efficacy against multiple malaria cases was estimated using negative binomial regression. Over the duration of follow-up, 744 of 1513 (49.1%) children and infants had at least 1 case of clinical malaria. Among children, vaccine efficacies were 42.7% (95% CI 25.7%, 55.8%, p<0.001) for the vaccine with booster group and 33.1% (95% CI 14.5%, 47.7%, p=0.001) for the vaccine group for first malaria case. Precipitation was significantly associated with increased malaria incidence, with each 1-inch increase in rainfall per month elevating the hazard of malaria by 12.6% (95% CI 9.6%, 15.6%, p<0.001) among children. There was no evidence of modification of vaccine efficacy by precipitation (p=0.85). The estimated numbers of cases averted were 9.4 (95% CI 2.5, 11.3) per 100 children per year in the vaccine group and 17.7 (95% CI 4.6, 32.0) in the vaccine with booster group, compared to the control group. In Malawi, an area of malaria mesoendemic, seasonal transmission, RTS,S vaccine efficacy was not modified by seasonal variation in precipitation. The WHO has selected Malawi as one of the sites to pilot roll-out of RTS,S to children. Our findings could be used by program implementers to help determine the most effective roll-out strategies.

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ANALYSIS OF THE CELLULAR IMMUNE RESPONSE IN C57BL/6 MICE TO FMP014 - A SELF-ASSEMBLING PROTEIN NANOPARTICLE BASED MALARIA VACCINE - DELIVERED IN ALF ADJUVANTS

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In the past decade, new developments in particle based vaccines and adjuvant systems have improved antigen presentation and immune system stimulation. These particle based vaccine platforms show great potential due to their ability to present high-density repetitive epitopes on the surface of the particle, which facilitates the presentation and processing of antigens by the innate and adaptive immune system. We have previously

reported promising results with the use of a self-assembling protein nanoparticle (SAPN) malaria vaccine that displays epitopes from the *Plasmodium falciparum* circumspozorzoite protein (PfCSP). These studies have shown that this vaccine platform induced long-lived and protective immune responses against infection in a mouse model incorporating an otherwise lethal challenge with a transgenic *P. berghei* parasite containing the full-length PfCSP gene (Tg-Pb/PfCSP). We have made additional modification of the PfCSP based SAPN particle and produced under cGMP conditions the falciparum malaria protein 14 (FMP014). FMP014 contains improved antigenic display of the αTSR and NANP repeat regions in addition to several CD4+, CD8+ and universal TH epitopes. We have combined the FMP014 antigen with several new liposome based adjuvant formulations referred to as ALF or Army Liposome Formulation. We have examined various FMP014/ALF vaccines with and without the addition of the aluminum hydroxide Alhydrogel® and the saponin QS21. These formulations produce strong humoral, cellular and protective immune responses in C57Bl/6 mice. Here we report on the cellular analysis of the FMP014/ALF vaccine formulations as determined by flow cytometry, ELISpot and multiplex cytokine arrays (Mesoscale Discovery). The FMP014/ALF vaccine formulations are currently undergoing safety and immunogenicity evaluation in non-human primates and are expected to enter a phase 1/2a study followed by a controlled human malaria infection (CHMI) challenge in 2017.

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ANTIBODY RESPONSES TO VACCINATION WITH *PLASMODIUM FALCIPARUM* APICAL MEMBRANE ANTIGEN 1 ARE BIASED TOWARD THREE CONSERVED IMMUNODOMINANT EPITOPES AND DO NOT MIMIC THOSE TO NATURAL INFECTION

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Plasmodium falciparum apical membrane antigen 1 (AMA1) is a highly polymorphic blood stage malaria vaccine antigen. The AMA1 subunit vaccine FMP2.1/AS02$_A$, tested in a Phase 2 clinical trial in Bandiagara Mali, elicited strong antibody responses, but limited and strain-specific protective efficacy against clinical malaria. When sera from FMP2.1-vaccinated children was probed on a whole-protein microarray containing 263 variants of the AMA1 ectodomain, anti-AMA1 antibody titers increased several-fold, regardless of AMA1 sequence. We hypothesized that the vaccine was biased towards cross-reactive, highly immunogenic epitopes that saturated the signal, obfuscating the smaller, strain-specific, response. To test this, we used sera from 20 of the same Malian children before and 90 days after AMA1 or control vaccination to probe a diversity-reflecting, ultra-dense, small linear peptide array, created using the same AMA1 sequences as the whole protein array, and an additional 68 AMA1 sequences derived from publically-available databases. The array was constructed using unique 16 amino acid long peptides covering the entire length of AMA1 and overlapping by 15 amino acids. Sera from AMA1-vaccinated children showed an increase in antibody titers at three conserved epitopes several-fold higher than control sera over the same time period. Sera from controls who experienced symptomatic malaria illness during the 90 days of follow-up had elevated titers of antibodies targeting different AMA1 epitopes; including epitopes that fell outside the AMA1 vaccine sequence. The high and broad vaccine-induced seroreactivity seen on the whole-ectodomain protein microarray can be attributed to three conserved and cross-reactive immune-dominant epitopes identified by the peptide array. While seroreactivity to tiled, linear peptides may not pinpoint the location of functional conformational epitopes, it may be useful to test whether the patterns acquired after malaria parasite exposure match antibody signatures generated by vaccines.

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LESSONS LEARNED FROM THE MANAGEMENT OF THE INVESTIGATIONAL PRODUCT DURING PHASES 1B &2B MALARIA VACCINES TRIAL IN BURKINA FASO

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An effective malaria vaccine will be a powerful tool that will contribute to reduce malaria burden. We report here experiences gathered in managing investigational product during many vaccines trial in CNRFP. All the trials were double-blind randomized controlled and have involved in total 925 children and 75 adults who have received three doses of either the malaria vaccine candidate or the control. The main storage of study vaccines was in CNRFP headquarters with frequent shipments to the two field sites, namely Balonghin and Banfora located respectively at around 45 minutes and 6 hours drive where participants were immunized. More than 2000 doses of malaria vaccine and 4000 doses of control were received from the various sponsors. Logistical constraints were increased since in some trials, the two vaccines required different condition of storage: Between +2°C and 8°C for control and - 65°C or -20°C for the malaria vaccine. In addition, the two vaccines were different in colours. Strategies for masking were used to keep the blinding for the subject and the vaccinator. We have experienced three temperature deviations of a cumulative duration of 13 hours. Main causes of deviation were material failure and power failure. Vaccines quality was not affected. More than 3500 doses were given with less than 3% of doses lost. In conclusion, despite many challenges, it was possible to ensure that vaccines were managed in accordance with international standards that protect the safety and well-being of trial participant in an African setting with resources limitation constraints.

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BIVALENT CONJUGATE VACCINE TO BLOCK MALARIA TRANSMISSION AND TYPHOID FEVER

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Conjugation of bacterial polysaccharide to a carrier protein is an effective tool to overcome poor immunogenicity of polysaccharide vaccines and to transform the T-cell independent immune response to T cell dependent one. Recent studies evaluating conjugates of Vi polysaccharide from Salmonella Typhi (Vi) to carrier proteins revealed the remarkable finding that conjugation to Vi may enhance immunogenicity of proteins in certain instances. Based on this observation, we have explored the possibility of generating a bivalent vaccine against malaria and typhoid fever, two diseases co-endemic in many parts of the world with high levels of morbidity and mortality, by conjugating malaria antigens to Vi, an approved vaccine for typhoid fever. A malaria Transmission Blocking Vaccine (TBV) that targets the mosquito stages of the parasite is being pursued as a product to interrupt transmission and contribute to elimination. Our laboratory has been evaluating a number of TBV antigens as chemical conjugates with protein carriers to enhance immunogenicity.

Here we describe our efforts to develop a bivalent vaccine consisting of TBV antigen conjugated to Vi to block malaria transmission and typhoid fever. We synthesized a number of conjugates of Vi polysaccharide with Pfs25, a TBV antigen, and evaluated their immunogenicity in mice. Our results showed that chemical conjugation results in enhancement of antibody responses against both Vi polysaccharide and Pfs25 compared to un-conjugated Vi and Pfs25. This increase in antibody titer was also found to be dependent on the conjugation method used for the synthesis. Functional studies using Standard Membrane Feed Assay showed enhancement of functional activity consistent with the increase in antibody titer. This Vi-malaria antigen conjugate concept will be further developed and tested for efficacy in other animal models as well as in human clinical trials if warranted.

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IMPACT OF THE ADDITION OF A SIGNAL SEQUENCE ON THE IMMUNOGENICITY OF A MULTI-STAGE VACCINE AGAINST *PLASMODIUM VIVAX* DELIVERED BY A NOVEL RECOMBINANT SIMIAN ADENOVIRUS VECTOR

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Plasmodium infections still pose a serious threat to public health worldwide. Several vaccine candidates have been tested but none of the formulations has been able to induce both robust cellular and humoral responses. Here, we describe a proof-of-concept study testing the effect of the inclusion of the signal peptide leader sequence, derived from the murine immunoglobulin kappa light chain, on the immunogenicity of a multistage malaria vaccine delivered as a transgene using the simian adenoviral vector serotype 36 (SAd36). The adenoviral transgene included the signal sequence in frame with the coding sequences of two *P. vivax* chimeric recombinant proteins previously developed by our group: PvRMC-CSP and PvRMC-MSP1, which targets the pre-erythrocytic antigen circumsporozoite protein (CSP) and the erythrocytic stage antigen merozoite surface protein 1 (MSP-1) respectively. Comparative experiments were conducted in mice using the recombinant vectors with, or without the signal sequence and heterologous adenoviral prime-protein boost regimens. Mice immunized with the adenoviral vector that included the signal sequence exhibited significantly higher antibody titers after the priming and significantly higher levels of IgG2a when compared to mice receiving the adenoviral vector without the signal sequence. The cellular response was also improved by the addition of the signal sequence as T cells produced higher levels of IFN-γ, TNF-α, and IL-2 upon *ex vivo* stimulation with the *P. vivax* chimeric proteins. Overall, our results demonstrate that the addition of a signal sequence is able to increase the immunogenicity of malaria vaccines delivered through adenoviral vectors.

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ANTIBODY RESPONSES TO HEPATITIS B SURFACE ANTIGEN FOLLOWING ADMINISTRATION OF RTS,S/ASO1E TO HIV-INFECTED AFRICAN INFANTS AND CHILDREN

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The RTS, S/AS01E malaria vaccine has been evaluated for efficacy, safety and immunogenicity in infants and children in sub-Saharan Africa. It consists of sequences of the circumsporozoite (CS) protein and hepatitis B surface antigen (HBsAg), making it also a hepatitis B vaccine. During a Phase III trial(NCT01148459) conducted at two centers in Kenya from July 2010 to May 2013, the vaccine was evaluated for safety and immunogenicity against the CS protein and HBsAg. Two hundred infants and children aged 6 weeks to 17 months who had HIV stage 1 or 2 disease, were randomized in a ratio of 1:1 to receive either the RTS,S/AS01E or rabies control vaccine each administered in 3 doses 1 month apart. Study participants received Hepatitis B vaccine prior to or during the study according to the Kenya vaccination schedule where it is administered at 6, 10 and 14 weeks of age. Anti-HBs antibody titers were measured prior to vaccination, 1 month post Dose 3 and 12 months post Dose 3 and the percentage of subjects with seroprotective levels of anti-HBs (10 mIU/ml and 100 mIU/ml) determined. Based on a threshold of 10mIU/ml, at baseline 57.1% (95%CI: 45.4-68.4) of individuals on the RTS,S/AS01E arm and 54.8% (95%CI: 42.7-66.5) of individuals on the rabies vaccine arm had seroprotective titers of anti-HBs with anti-HBs Geometric Mean Titers (GMTs) of 24.1 mIU/ml (RTS,S/AS01E arm) and 19.2 mIU/ml(rabies arm). One month post-dose 3, 100% (95%CI: 95.1-100) of subjects in the RTS,S/AS01E arm and 52.3% (95%CI: 39.5-64.9) in the rabies arm were seroprotected for anti-HBs with anti-HBs GMTs of 13,637.6 mIU/ml (RTS,S/AS01E arm) and 19.9 mIU/ml (rabies arm). Twelve months post-dose 3, 100% (95%CI: 94.9-100) of subjects in the RTS,S/AS01E arm and 39.1% (95%CI: 27.1-52.1) in the rabies arm were seroprotected for anti-HBs with anti-HBs GMTs of 2,294.8 mIU/ml(RTS,S/AS01 arm) and 11.8 mIU/ml (rabies arm).The RTS,S/ASO1E malaria vaccine generated a strong immune response to Hepatitis B Surface antigen. Therefore, it could have the additional benefit of providing protection against Hepatitis B to children who have not been fully protected through routine vaccination programmes.

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A CLINICAL TRIAL TO EVALUATE THE SAFETY AND INFECTIVITY OF DIRECT VENOUS INOCULATION OF ASEPTIC, PURIFIED, CRYOPRESERVED *PLASMODIUM FALCIPARUM* (7G8 AND NF54) SPOROZOITES IN MALARIA-NAÏVE ADULTS IN BALTIMORE, USA

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Controlled human malaria infection (CHMI) by the bites of infected *Anopheles* mosquitoes is a safe and reproducible method to assess malaria vaccine and drug efficacy, but requires considerable capital and labor investment. Direct venous injection (DVI) of aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) NF54 sporozoites (SPZ) (Sanaria® PfSPZ Challenge) closely mimics CHMI by mosquito bite, simplifying malaria vaccine and therapeutics testing. As multiple, genetically diverse *Plasmodium* strains with varying susceptibility to antimalarial drugs circulate in endemic areas, testing malaria vaccines and therapeutics using a single parasite strain is not optimal. Development of additional strains for CHMI by DVI would facilitate vaccine and therapeutics testing against parasites that represent strains circulating in nature and that are heterologous to vaccine strains. Pf7G8 is a Brazilian strain with a divergent genomic sequence from PfNF54. Unlike PfNF54, Pf7G8 parasites are resistant to chloroquine, but both are susceptible to atovaquone-proguanil. This single center, randomized controlled human study aims to identify the dose of Pf7G8 SPZ administered by DVI that achieves 100% infection rates. Thirty-six participants will be randomized to one of five groups. Four groups receive escalating doses of Pf7G8 (800, 1600, 3200 and 4800 SPZ) and one control group receives standard dose PfNF54 that infects 100% of malaria-naïve participants (3200 SPZ). Participants will be monitored clinically and malaria positivity will be determined by qPCR. Study objectives compare increasing doses of 7G8 versus NF54 with respect to safety and reactogenicity, infectivity, and time to patency. The study is planned for summer 2016 and initial results will be presented. This clinical trial will serve to standardize optimization testing of other strains slated for CHMI by DVI.

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EVALUATION OF THE SAFETY AND IMMUNOGENICITY OF NANOPARTICLE FORMULATIONS WITH RECOMBINANT *PLASMODIUM FALCIPARUM* TRANSMISSION-BLOCKING ANTIGEN PFS25

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An important consideration for vaccine development is the identification of an appropriate adjuvant, which is capable of eliciting an optimal protective immune response without raising any safety concerns. The objective of this work was to screen various nanoadjuvants and to evaluate the toxicity and immunopotentiating ability *in vitro* and *in vivo*. We compared Alum, nanoemulsions (NE), poly (lactic-co-glycolic acid) (PLGA), gold nanospheres (GNP) and gold nanoprism (GNPR) formulated with a malaria vaccine candidate, CHrPfs25, which has been extensively investigated in our lab. We assessed *in vitro* cytotoxicity using human monocytic cell line (THP-1, bone marrow derived dendritic cells (BMDC) and hepatic carcinoma cell line (HepG2). Cells were exposed to the nanoadjuvanted vaccine for 24h, and an MTT assay was used to assess the viability of cells. THP-1 and BMDC did not demonstrate any cytotoxicity. HepG2 cells showed varying levels of mild cytotoxicity. GNP was most the biocompatible and PLGA exhibited more cytotoxicity. CHrPfs25 formulated with different nanoadjuvants were also evaluated in mice for *in vivo* cytotoxicity and immune-potentiating effects. The results revealed that CHrPfs25 delivered with GNP elicited stronger humoral responses than other groups. There was no apparent toxicity associated with the administration of these formulations. PLGA elicited the lowest response. In an attempt to understand the action of these adjuvants *in vivo*, mice were immunized intramuscularly with different CHrPfs25-nanoadjuvanted formulations followed by assessment of DC and Macrophageϕ ((antigen presenting cells) infiltration at site of injection, sera cytokine and blood profiles after 3, 7 and 14 days of immunization. The results from these studies will be presented.

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EVALUATING THE POTENTIAL TO TRANSMIT MALARIA FROM HUMANS TO MOSQUITOES DURING CONTROLLED HUMAN MALARIA INFECTION WITH *PLASMODIUM FALCIPARUM* AND *P. VIVAX*

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The controlled human malaria infection (CHMI) model has been used successfully to assess efficacy of drugs and vaccines targeting the pre-erythrocytic and blood stages of malaria infection. However, existing models are yet to be used to assess the ability of interventions to interrupt transmission of malaria from humans to mosquitoes. Such a model would be an invaluable tool for selecting the most promising transmission blocking interventions (TBIs) for further evaluation. Our ongoing work uses the induced blood stage malaria (IBSM) model with either *Plasmodium falciparum*- or *P. vivax*- infected red blood cells to initiate blood stage infection in malaria naïve volunteers. Volunteers are subsequently treated with licensed or experimental antimalarials to assess drug activity. As a secondary aim, we are investigating if the gametocytes that appear in peripheral circulation during these CHMI studies, either before drug treatment in the case of P.v infection or after drug treatment in the case of P.f infection, can be transmitted to *Anopheles* mosquitoes. During these studies the development of parasitemia and gametocytemia are monitored in the volunteers by qPCR and qRT-PCR, respectively. Following detection of gametocytes, transmission studies are performed using both direct feeds on volunteers and membrane feeding assays on venepuncture blood. Mosquito infection is then determined 7-10 days post feeding assay by detecting oocysts in the mosquito midgut. In recent clinical trials we have achieved successful transmission of both P.f and P.v at low levels. Current work now aims to optimise this system to enable assessment of its potential for evaluating TBIs. Analysis of specific quantitative biomarkers is being undertaken to improve our understanding of factors that may contribute to transmission efficiency, including male:female gametocyte sex ratios and gametocyte commitment, and in addition we are aiming to optimise the susceptibility of vector mosquitoes to infection. This work will contribute to the development of a reproducible model for rapid selection of effective transmission-blocking drugs and vaccines.

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CLINICAL DEVELOPMENT OF A VAR2CSA-BASED PLACENTAL MALARIA VACCINE PLACMALVAC: DECRYPTION OF THE ANTIBODY ACQUISITION AGAINST THE VACCINE CANDIDATE ID1-ID2

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Placental malaria (PM) is an important cause of maternal anemia, stillbirth and delivery of low birth weight babies, the latter representing a major risk factor for infant mortality in Africa. Many studies have underlined the key role of the parasite protein VAR2CSA in placental malaria (PM), the leading PM vaccine candidate. A specific immune response against VAR2CSA is acquired during the first pregnancies and reduces the harmful effects of placental malaria during subsequent pregnancies. This has led to the development of a candidate vaccine by an EU-funded consortium (PlacMalVac project) which is currently under Phase I trial in Germany and Benin.As part of the PlacMalVac project, we quantified anti-Id1-Id2 IgG and subtype responses to the VAR2CSA subunit vaccine candidate using ELISA in a cohort of Beninese pregnant primigravidae enrolled before the beginning of pregnancy. Clinical and parasitological data were collected monthly from 37 nulligravid women who became pregnant and followed through to delivery. Similar antibody measurements were performed in samples from a sub-cohort of 470 pregnant women of different parities who were followed up throughout pregnancy in the stoppam study. Preliminary analysis shows that antibody levels are dependent on pregnancy, parity status, and are associated with the occurrence of infection during pregnancy. These analyzes highlight the key role of anti-Id1-Id2 IgG3 in protection against placental malaria.

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ANTIBODIES TO PLANT-PRODUCED *PLASMODIUM FALCIPARUM* SEXUAL STAGE PROTEINS EXHIBIT TRANSMISSION BLOCKING ACTIVITY

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Transmission blocking vaccines are considered a critical component in the overall strategy for control and eventually elimination of malaria world-wide. Sexual-stage proteins expressed by *Plasmodium falciparum*, Pfs230 and Pfs25, are the main transmission blocking antigens moving through clinical trial development. Antibodies generated upon vaccination with either of these results in interruption of sporogonic development in the mosquito, and transmission to the next host. Using a plant based transient expression system, we have produced Pfs25 and Pfs230 fused to various carrier proteins in *Nicotiana benthamiana*, purified and characterized the proteins, and evaluated the vaccine candidates in animal models for generation of transmission reducing antibodies (TRA)/ transmission blocking antibodies (TBA). The Pfs25 and Pfs230 vaccine candidates are expressed at high levels, and induced TBA that persist up to 6 months post immunization. These data demonstrate the potential of the new malaria vaccine candidate and also support feasibility of expressing *Plasmodium* antigens in a plant-based system.

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QUANTIFICATION OF BED-NET LOSS AND LEAKAGE FOLLOWING A MASS-DISTRIBUTION CAMPAIGN ON BIOKO ISLAND USING THE CAMPAIGN INFORMATION MANAGEMENT SYSTEM (CIMS)

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Between December 2014 and June 2015, the Bioko Island Malaria Control Project (BIMCP) distributed 149,287 long lasting insecticidal nets (LLIN) to 61,000 households on Bioko Island, achieving an Island-wide coverage of at least 1 LLIN per household of 87%. Of the 87% of households contacted who received a net, universal coverage (at least one net per two people) was achieved in 89% of them, for an Island-wise universal coverage of at least 77%. The BIMCP planned and implemented the distribution campaign through a tablet-based Campaign Information Management System (CIMS) that contains a georeferenced listing of all households on the Island, linked to a unique household identifier. Using the CIMS, data were collected on household size, number of pre-existing nets, and number of nets distributed. Between August and October of 2015, approximately 7 months after the mass distribution, the BIMCP carried out a Malaria Indicator Survey (MIS), taking a representative sample of all communities in the Island. The MIS included questions about bed-net ownership and usage. The MIS showed that net ownership had dropped by 22% between the time of distribution and the 2015 MIS, with 69% of households reporting owning at least one LLIN in the MIS. Universal coverage dropped by 45%, with only 42% of households reporting having at least one net per every two people. Using the geo-referenced unique household identifier, we were able to compare net ownership in 4,992 households. Fifty seven percent of these households reported having at least one less net at the time of the MIS than were distributed during the distribution campaign, and 34% reported at least two fewer nets. While many households reported a loss of nets, others reported a gain of nets. An in-depth analysis of the net code inscribed during the distribution and reported in the MIS, which reveal the original community LLINs were distributed to, will be conducted to investigate possible redistribution of nets. Additionally, results from the 2016 MIS will be analyzed to quantify net loss one year following the mass-distribution and better evaluate the characteristics of households with net gain and loss.

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DYNAMICS OF ENTOMOLOGICAL INOCULATION RATES FOLLOWING INDOOR RESIDUAL SPRAYING IN MALI

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Entomological monitoring is used to assess the impact of indoor residual spraying (IRS) on entomological indicators such as the entomological

inoculation rate (EIR). We assessed the impact of IRS on EIR in sprayed and un-sprayed areas over four years in one district in Mali. *Anopheles gambiae* s.l. were sampled using human landing catches. Baseline data (pre-IRS) were collected at the beginning of the high malaria transmission season one week before spraying. Post-spray campaign follow-up data were collected during the high malaria transmission season for two months in 2012, three months in 2013 and 2014, and five months in 2015. Intervention areas were sprayed with bendiocarb in 2012, 2013 and 2014 and pirimiphos-methyl (300CS) in 2015. An enzyme-linked immunosorbent assay estimated the proportion of mosquitoes positive for *Plasmodium falciparum* sporozoites. In 2012, the pre-spray EIR/night was 0 and 2.64 in the intervention and control areas, respectively. Post-spray EIR was 0.35 in the intervention area and 8.88 in the control area. In 2013, pre-spray EIR/night was 0.60 in the intervention area and 0.97 in control area. Post-spray EIR was 0.49 in the intervention area and 1.47 in control area. In 2014, pre-spray EIR/night was 0.58 in the intervention area and 3.32 in control area. Post-spray EIR was 0.27 in the intervention area and 2.31 in the control area. In 2015, pre-spray EIR/night was 0.75 in the intervention area and 0.84 in the control area. Post-spray EIR was 0.89 in the intervention area and 3.53 in the control area. IRS was effective in maintaining or reducing *An. gambiae* s.l. EIR, which is a measure of malaria transmission. Further malaria case reporting is needed to monitor the impact of IRS.

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USE AND USER CHARACTERISTICS OF INTACT OR "TOO TORN" INSECTICIDE-TREATED MOSQUITO NETS IN TANZANIA

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Sleeping under insecticide-treated mosquito nets (ITNs) is one of the most effective ways for people in malaria-endemic countries to protect themselves against malaria. As most of Sub-Saharan Africa is moving towards universal coverage of the population with mass ITN distribution campaigns, net use rates have been increasing. However, there is still a lack of data on user characteristics, especially of nets with different degrees of physical damage. Two cross-sectional household surveys were carried out in eight districts in Tanzania in 2014 and 2015 to collect data on net ownership, use and physical condition (hole surface area and proportionate Hole Index) combined with household member characteristics. The surveys were conducted in 2,944 households with 15,627 household members and 4,793 used mosquito nets. This data will be analysed to assess who uses mosquito nets of what quality under different ITN ownership scenarios. Results will be presented on characteristics of ITN users (e.g. age, gender, pregnancy status, role in household), whether certain user groups are more likely to sleep underneath "good" or "too torn" nets and whether household ownership of ITNs has any effect on who uses nets of different qualities. This study provides important information, to our knowledge for the first time, on who uses mosquito nets of varying physical integrity, and whether this depends on the access of household members to ITNs. These factors will help malaria policy makers target the right audiences when developing behavioural communication strategies and net replacement campaigns.

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ENTOMOLOGICAL INDICATORS OF MALARIA TRANSMISSION AFTER IRS WAS DISCONTINUED: FINDINGS FROM SAVELUGU NANTON DISTRICT AND ITS IMPLICATIONS FOR MALARIA CONTROL IN GHANA

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Savelugu Nanton District (SND), in the northern region of Ghana, was a beneficiary of the President's Malaria Initiative (PMI) supported indoor residual spraying (IRS) program between 2008 and 2014. Entomological monitoring data showed that parity rates of *An. gambiae* s.l. had been reduced from 44.8% in 2011 to 28.1% by 2014. Sporozoite infection rates ranged between 0.23% and 1.89% between 2011 and 2012, and remained significantly low at a level that could not be detected for two consecutive years (2013 and 2014). IRS was withdrawn from the district after the 2014 IRS campaign due to low coverage and indications of emerging insecticide resistance.Monthly entomological data from three sentinel communities in the district revealed a significant increase in parity, sporozoite rate and entomological inoculation rates (EIR) of the local vector species just one year after the withdrawal of IRS. Parity rate of *An. gambiae* s.l. increased from 28.1% in 2014 to 51.2% in 2015 ($p<0.0001$) after IRS withdrawal. The sporozoite infection rate increased from a level that could not be detected for two consecutive years (2013 and 2014) to 1.10% (4/361 mosquitoes analyzed). Consequently, the EIR increased from undetectable levels in 2013 and 2014 to 14.7 infective bites/man/year in 2015. In Bunkpurugu Yunyoo District, where spraying continued without interruption, EIR declined from 3.3 ib/m/yr in 2014 to 0.82ib/m/yr in 2015. The results from the surveys show that the IRS program maintained low levels of the two most important indicators of malaria transmission: parity and infectivity rates of the malaria vectors. However, there was a significant resurgence following withdrawal of IRS from the area. Epidemiological data is needed to understand the impact of IRS on malaria transmission.

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A COMPARISON OF THE EFFECTIVENESS OF BEHAVIOR CHANGE COMMUNICATION (BCC) PLUS REPAIR KITS AND BCC ALONE IN PROMOTING REPAIR OF LONG-LASTING INSECTICIDAL NETS IN BENIN

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We compared strategies to increase net durability in 2014. Three groups of 300 households (HH) were randomly assigned to two intervention and one control arm. Arm 1 received behavior change communication (BCC) messages; Arm 2 received BCC plus a net repair kit; Arm 3 was the control. Twelve villages in southeastern Benin were enrolled. Community health workers delivered BCC messages about preventing damage to long-lasting insecticidal nets (LLIN) caused by fire or sharp objects, and

promoting repair as soon as holes appeared. Data were collected from all HH at 4-5 month intervals for 20 months. Net damage was measured using the WHO Proportional Hole Index (pHI). At 20 months the overall HH dropout rate was 21% (17% for Arm 1, 23% for Arm 2 and 24% for Arm 3). Only one of five control HH (Arm 3) reported hearing messages about net care or repair. Net attrition (LLIN not available to sleep under) was significantly lower in Arms 1 and 2 (9%), than in Arm 3 (16%) ($p<0.0001$). LLIN use among children under five years was higher among those receiving BCC and repair kits (83%) than those receiving only BCC (73%) ($p=0.02$). Intervention Arms 1 and 2 reported more net use (73% and 83%, respectively) than the control arm (63%) ($p<0.0001$). Frequent LLIN washing (>1 wash/3 months) resulted in reduced net integrity in the control group. Reduced insecticide activity was also more common in the control arm (78%) than Arm 1 (69%) and Arm 2 (56%) ($p<0.0001$). The proportion of nets without holes was significantly higher in Arm 2 (53%) than in Arm 3 (38%) ($p=0.019$), but no difference was observed in the prevalence of holes between Arm 1 (41%) and the control arm (38%). Nets in Arms 1 and 2 showed more signs of repair (57% and 58%, respectively) than controls (22%) ($p<0.0001$). The proportion of nets with large and/or numerous holes (pHI >63) was significantly lower in Arm 1 (13%) and Arm 2 (9%) than in Arm 3 (36%) ($p<0.0001$). All LLIN in all three arms had good insecticide retention measured by x-ray fluorescence and WHO cone test. BCC messaging significantly increased care and repair practices in intervention villages in Benin. Whether these practices can prolong LLIN durability requires further study.

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DRY SEASON MALARIA TRANSMISSION REDUCES THE IMPACT OF SEASONAL INDOOR RESIDUAL SPRAYING IN BENIN

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Indoor residual spraying (IRS) in Benin is based on a single round of spraying to coincide with the annual period of highest transmission (peak vector density). It is assumed that during the dry season, when IRS is not done, vectors should be few and the level of malaria transmission low. In this study, we tested this assumption by measuring the intensity of malaria transmission during the peak transmission and low transmission (dry season periods) using entomologic measures. We assessed *Anopheles* population dynamics over a five year period by measuring human-biting behavior and entomological (*Plasmodium falciparum* sporozoite) inoculation rates during periods when IRS was done and during the dry season when it was not carried out. A total of 3,752 *Anopheles* (*An. gambiae*, *An. coluzzii* and *An. funestus*) were collected. During the period of IRS impact (June-October: rainy season) *An. gambiae* was the most abundant species (87.8% in Atacora and 94.3% in a similar comparison area, not under IRS). In dry season, when IRS insecticidal effect was not operating (November to May), the percentage of *An. gambiae* dropped to approximately 20%, but two other species, *An. coluzzii* and *An. funestus* made up 68.4% and 12%, respectively, of the *Anopheles* caught). More importantly, however, transmission of *P. falciparum*, continued, estimated to be approximately three infective bites per human per month for *An. gambiae* s.s. and one infective bite per human per month for *An.* funestus, which was higher than expected for a period when the most *An. gambiae* s.s. breeding sites were assumed to dry up. The abundance of *An. coluzzii* and *An. funestus* in dry season probably owes its origin to breeding sites maintained by permanent and semi-permanent streams in the Atacora foot hills.

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EFFECT OF IVERMECTIN ON *PLASMODIUM VIVAX* IN ITS INTERACTION WITH *ANOPHELES AQUASALIS*

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The use of insecticide-treated nets and indoor residual insecticides targeting adult mosquito vectors is a key element in malaria control programs. However, mosquito resistance to the insecticides used in these applications threatens malaria control efforts becoming in a major public health issue. Alternative methods like Ivermectin administration to humans has been suggested as a possible vector control to reduce *Plasmodium* transmission. *Anopheles aquasalis* isa competent vector for *P. vivax* and it has been responsible for various malaria outbreaks. This study analyzed the effect of Ivermectin on vector competence of *An. aquasalis* for *P. vivax* using two experimental protocols: (A) One single Ivermectin dose (200 µg/mL) was taken by volunteers and blood samples were drawn at distinct times. *An. aquasalis* was infected by membrane feeding with different concentration of these blood samples mixed with *P. vivax* from malaria patients. Seven days after the infective bloodmeal, the mosquitoes were dissected to check the oocyst presence and the infection rate. (B) Additionally, the *in vitro* effect of the addition of Ivermectin on cultivated *P. vivax* was observed. Ivermectin significantly reduced the proportion of *An. aquasalis* that developed oocysts (40ng/mL concentrationor plasma 4 h, but with the metabolized Ivermectin on 5, 10 and 14 d post-treatment it was not reduced ($p=0.06$; $p=0.91$; $p=0.80$ respectively). *Plasmodium vivax* infection was significantly reduced in the survived *An. aquasalis* that ingested Ivermectinin the 40ng/mL concentration and plasma 4h post-treatment. In the *in vitro* cultures, Ivermectin (plasma 4 h concentration) significantly affected the *P. vivax* asexual development, reducing the number of schizonts (50% of inhibition). In conclusion, Ivermectin reduces the infection rate of *P. vivax* in *An. aquasalis* and increased the mortality of mosquitos. These findings support the idea that Ivermectin is useful to reduce *P. vivax* transmission in endemic areas.

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DETECTION OF *PLASMODIUM FALCIPARUM* INFECTION IN *ANOPHELES SQUAMOSUS* IN AN AREA TARGETED FOR MALARIA ELIMINATION, SOUTHERN ZAMBIA

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Southern Zambia is the focus of strategies to create malaria-free zones. Interventions being rolled out include reactive test and treat strategies ('Step D') and distribution of insecticide-treated bed nets. Step D involves the identification of index cases, i.e. non travelers who test positive for malaria at health facilities, who are subsequently followed up at their homes for malaria screening and treatment of all residents and of neighboring households. In 2015 in Macha, Choma District, mosquitoes were collected monthly by light trap from Step D homesteads, set both indoors next to occupied bed nets and outdoors next to goat enclosures to study vector foraging patterns around clusters of malaria cases. Anopheline mosquitoes were identified to species using molecular methods and *Plasmodium falciparum* infectivity was determined by ELISA and real time qPCR methods. Comparing indoor collections of anophelines from Step D households to those of a random selection of households within the community, species composition was similar with domination of the vector *Anopheles arabiensis*, however household densities were

four fold higher in Step D houses suggesting local and focal transmission. Catches from outdoor traps were nine times greater than indoor collections with more than 60% identified as *An. squamosus*. Analysis of a subset (n=1006) of anophelines were analysed by ELISA for malaria sporozoites. Seven were found positive, of which four were confirmed as harboring parasites by qPCR. All seven specimens were caught outdoors. Six were morphologically identified as *An. squamosus* and one as *An. coustani*. Parasite-positive specimens as well as a subset of *An. squamosus* specimens from either the same study or archive collections underwent sequencing of the mitochondrial COI gene. Maximum parsimony trees indicated presence of at least 2 clades of *An. squamosus* with infectious specimens falling in each clade. The single infectious specimen identified morphologically as *An. coustani* could not be matched to reference sequences. This is the first report from Zambia of infections in *An. squamosus* and from outdoor collections.

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FIELD TESTING OF A PYRETHROID QUANTIFICATION KIT (PQK) IN TANZANIA - AN EASY-TO-USE TOOL FOR MONITORING THE QUALITY OF INDOOR RESIDUAL SPRAY CAMPAIGNS

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Insecticide treated nets (ITN) and indoor residual spraying (IRS) are two of the primary methods of malaria prevention in Africa. In order for these methods to be effective it is essential that adequate concentrations of insecticide are present on nets and wall surfaces to kill mosquitoes. There is no easy assay to quantify insecticide levels without expensive laboratory equipment and procedures. To address this, LSHTM has developed a simple field-applicable kit for monitoring pyrethroid residues on insecticide-treated nets- the Pyrethroid Quanitification Kit (PQK)-which can be adapted to other types of treated surfaces. During the initial trial the PQK kit was calibrated against a variety of sprayed surfaces and with different concentrations of lambdacyhalothrin before being taken into the field. Mosquito cone bioassay was conducted to show whether the surface concentrations of insecticide detected by the PQK were sufficient to kill a susceptible strain of mosquitoes. Houses in six villages were visited 3 months after IRS had been conducted in Muleba, Tanzania. The samples were analysed in the field using a handheld spectrophotometer. In each house, five areas of the wall were examined to give an indication of insecticide distribution and within-wall variation. Results showed that the actual spraying results differed from expectation. Preliminary results showedthat only 28% of houses had all rooms sprayed, leaving 72% of housespartially sprayed, and insecticide concentration varied dramatically across sprayed walls. The PQK is an easy to use quality assurance tool for monitoring of pyrethroid application rates and improving the quality of IRS campaigns.

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A COMPREHENSIVE ACCESS METRIC FOR ESTIMATING THE GAP IN INSECTICIDE TREATED NET USE CONDITIONAL ON ACCESS

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The complement of the proportion of people who use insecticide treated nets (ITNs) out of those who have access is a measure of the behavioural gap (BG) in ITN use. The updated Global Malaria Action Plan's access measure (UGMAPAM) is based on the assumption that two people can sleep under one ITN. However, often ITNs are shared by more than two people, and the number of people using an ITN can be larger than the UGMAPAM (resulting in a use : access ratio larger than one, and consequently a negative BG if not floored at zero), while there may still be people not using ITNs despite having access. The fact that the UGMAPAM-based use : access ratio is not a true proportion makes it unsuitable for standard statistical analyses. For the purpose of estimating the BG, we propose a comprehensive access measure (CAM), counting those who slept under an ITN as having access, as well as those who could have used spare spaces under an ITN, with one spare space for a single-occupied ITN and two spaces for an unoccupied ITN. The use : access ratio with the CAM as denominator is a true proportion, and its complement, the proportion of people who had access to a full person-size space under an ITN but did not use it, is an easy interpretable measure of the BG. We analyzed 85 Demographic and Health Surveys and 20 Multiple Indicator Cluster Surveys with specific information on mosquito nets from 44 countries over 2001-2015. An average of 46.2% (range: 15.1-71.8) of ITN users shared their net among three people or more. Compared to the CAM-based BG, the UGMAPAM-based BG (floored at zero) was on average 8.0 percent-points (range: 0-18.8) smaller, and the difference was largest at intermediate BG. We conclude that the use of the UGMAPAM-based BG has underestimated the potential for ITN use-stimulating interventions such as behavioural change communication and ITN improvements to increase acceptability.

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MALARIA UPSURGE FOLLOWING WITHDRAWAL OF INDOOR RESIDUAL SPRAYING IN NORTHERN UGANDA

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Uganda has registered significant declines in malaria prevalence in the recent past. However, concerns about maintaining the gains made are apparent. Starting 2009, indoor residual spraying (IRS) was implemented in 10 high burden districts in Northern Uganda resulting in suppressed transmission to very low levels. Based on this achievement and the country's deployment of long-lasting insecticidal nets universally in 2013 - 2014, IRS was withdrawn in November 2014. However, in March 2015 routine surveillance showed a marked increase in malaria cases and deaths suggestive of an epidemic in that region. We describe here the investigation and confirmation of the epidemic. Following reported increases in malaria cases, an epidemic preparedness and response team was constituted to investigate the upsurge in affected districts. A review of hospital records and malaria parasite testing of 11,000 blood smears collected between June-August 2015 was done to confirm the malaria case load. Mean indoor resting density of malaria vectors was done for entomological assessment. Reported malaria cases and slide positivity rate (SPR) were compared for the period during and after withdrawal of IRS. Out of 11,000 blood smears examined, 81% were positive for *P. falciparum*. Seventy two percent of hospital admissions were due to confirmed malaria. Based on hospital records, malaria incidence increased from 61 to 142 cases per 1,000 population and SPR from average 11% to 75% [difference 64%, CI:63.7-64.3] $p < 0.001$, before and after IRS withdrawal respectively. Entomological assessment indicated re-bound of vectors with mean indoor resting density of 4 female *Anopheles* mosquitoes per house visited. A dramatic increase in reported malaria cases, an SPR above the established epidemic thresholds confirmed

presence of a malaria epidemic in this region. Indoor Residual Spraying gains are fragile and can potentially be lost in the absence of a clear exit strategy.

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COVERING HOUSE EAVE GAPS AND CEILINGS WITH OLYSET® NET REDUCES RISK OF *PLASMODIUM FALCIPARUM* PARASITE INFECTION AMONG CHILDREN: A CLUSTER RANDOMIZED CONTROLLED TRIAL

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Long-lasting insecticidal bed nets have been widely used for reducing malaria cases. A persisting challenge is how to protect children who do not adhere to net use. This study examined whether covering house eave gaps and ceilings with a fabric made of Olyset®Net (ceiling nets) reduces risk of *Plasmodium falciparum* parasite infection among children under 10 years of age. Bed nets (Olyset®Net) were provided to cover all residents in the study area in western Kenya. Then, the area was divided to eight sub-areas, and four sub-areas were randomly selected for the treatment. Ceiling nets were installed in all houses in the selected sub-areas. The PCR-based pre-intervention infection rate of *P. falciparum* was 68.9%, and reduced to 27.3% (OR: 0.84; p<0.001) in the sub-areas covered with ceiling nets and bed nets. Similarly, the PCR-based infection rate significantly reduced from 60.9% to 44.9% in the sub-areas with bed nets only (OR: 0.94; p<0.001). The post-intervention infection rate in the sub-areas with ceiling nets was significantly lower than that in the sub-areas without them (OR: 0.45; p=0.031). While bed nets reduced risk of *P. falciparum* parasite infection, ceiling nets provided additional protection.

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REPELLENCY OF THREE ESSENTIAL OIL AND MAJOR CONSTITUENTS TO WILD ADULT *ANOPHELES KLEINI*

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Repellency of 20 plant essential oils to malaria main vector in the Republic of Korea (ROK), *Anopheles kleini*, was evaluated using skin direct contact bioassay. *An. kleini* showed the highest repellency to Pelargonium graveolens(Geranium oil)with EC50 value of 0.244 mg/cm2, followed by *Pinus sylvestris* (Pine oil) and *Cinnamomum camphora* (camphora oil) with EC50 values of 0.484 mg/cm2 and 0.862 mg/cm2. The lowest repellency of *An. kleini* was revealed from Clary sage oil with EC50 value of 4.665 mg/cm2. *An. kleini* did not demonstrated any repellency to Lemon, Orange, Neem, Cocount and Olive oil over 20 mg/cm2. Major repellent constituents of Geranium, Pine and Camphora oil were analyzed and identified using Mass-data, GC and GC-Mass. Major constituent of Geranium were β-citronellol (37.0%) and Camphora, 1,8-cineole (35.8%) and Pine, α-terpineol (39.5%). *An. kleini* showed higher repellency to β-citronellol and 1,8-cineole than to DEET and IR3535 and did not showed any repellency to sabinene and γ-eudesmol over 20 mg/cm2. Residual repellent time of 1,8-cineole and β-citronellol were 26 and 41 min, respectively and DEET, 84 min and IR3535, 102 min. In the light of global efforts to reduce the level of highly toxic synthetic repellents, the three essential oils and their major constituents described merit further study as potential biorepellents for the control of *An. kleini* populations .

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PREVALENCE OF SOME ENTEROPATHOGENS AMONG DIARRHOEIC AND APPARENTLY HEALTHY CHILDREN IN EKET AND IBENO, AKWA IBOM STATE, NIGERIA

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In this study, some intestinal bacterial pathogens involved in diarrhoea causation was investigated in Eket and Ibeno between October, 2013 to April, 2014. A total of 150 freshly - voided diarrhoeic samples of children attending Primary Health Care Centre, Eket and General Hospital, Ibeno, and 50 non - diarrhoeic samples were collected which served as controls. Standard bacteriological media and procedures were used in the identification of bacterial isolates. Antibiotic susceptibility test was done by standard procedures.The overall prevalence rates recorded for enteropathogens among children were 74% and 72% in Eket and Ibeno, respectively and the mean prevalence was 73%. Data obtained from the questionnaires given to subjects' mothers for socio - demographic information showed a high prevalence of enteropathogens in the following parameters examined; subjects within the age group of 7 - 12 months, subjects whose source of drinking water was stream, subjects' mothers in the civil service and self - employed groups, exclusively breastfed subjects. A decrease with increase in educational level of subjects' mothers was observed. The bacterial pathogens considered were *Escherichia coli*, Salmonella enteritidis, *Shigella dysenteriae* and Enterobacter species. *Escherichia coli* was the most prevalent with rates of 43.5% and 45% in Eket and Ibeno, respectively. Enteropathogenic *Escherichia coli* (EPEC) O26 and O111 were identified with an overall prevalence of 46.7%. In the antibiotic susceptibility test done, the organisms were mostly susceptibility to Ciprofloxacin and resistant to Cotrimoxazole. The study has revealed that the incidence of enteropathogens in children could be traced primarily to poor personal hygiene and faulty weaning practices. Therefore, a systematic effort to teach nursing mothers and children to practice good personal hygiene are the best approaches to reduction of the scourge of intestinal pathogens.

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DEFINING THE BURDEN AND EPIDEMIOLOGY OF SHIGELLOSIS IN RURAL ASEMBO, WESTERN KENYA, 2007-2014

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Shigella is a leading cause of diarrhea and dysentery in low- and middle-income countries. Population-based data on the Shigella burden in African settings are very limited. We describe the incidence of medically attended shigellosis in a population of ~25,000 who reside ≤5 kilometers from a surveillance clinic in Asembo, rural western Kenya that provides free medical care. From January 1, 2007-December 31, 2014, stools and demographic data were collected from all patients presenting with diarrhea (≥3 loose stools within 24 hours). The specimens were cultured and isolates identified biochemically and confirmed by serotyping. Participants were visited at home biweekly and queried about acute illnesses and care-seeking. We calculated the incidence of Shigella infections per 1000 person-year-observation (pyo); we adjusted for the proportion of diarrhea cases with no stool sample, and for the proportion of diarrhea cases reported during household visits that sought care at a health facility other than the surveillance clinic. A total of 11,775 cases of diarrhea presented to the clinic during the study period. Of these a stool specimen was collected from 1,658 (14%), and Shigella was isolated from 418 (25%). The overall adjusted incidence rate was 11.1/1000 pyo;

among those aged <5 and ≥5 years, it was 9.1 and 11.2 per 1000 pyo respectively. Peaks in incidence were seen in young children (<12months 15.0/1000 pyo, 12-23 months 12.7/1000 pyo) and older adults (35-49 years 19.0/1000 pyo, ≥50 years 23.7/1000 pyo). The annual adjusted incidence was highest in 2011 (13.8/1000 pyo) and lowest in 2013 (7.4/1000 pyo). *Shigella* species isolated included: *S. flexneri* 251 (60%), *S. dysenteriae* 57 (14%), *S. sonnei* 42 (10%), *S. boydii* 39 (9%) and non-typeable 29 (7%). We found an important burden of *Shigella*, particularly *S. flexneri*, among children and adults, with both age extremes most heavily affected. Prevention strategies, such as access to safe water and sanitation and future vaccines, should address disease burden across all age groups.

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RISK FACTORS ASSOCIATED WITH TYPICAL ENTEROPATHOGENIC *ESCHERICHIA COLI* INFECTION AMONG CHILDREN <5 YEARS OLD WITH MODERATE-TO-SEVERE DIARRHEA IN RURAL WESTERN KENYA, 2008-2012

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Typical enteropathogenic *Escherichia coli* (tEPEC) infection is a major cause of diarrhea and contributor to mortality in children <2 years old in developing countries. Limited data are available on risk factors associated with tEPEC infection in young children. Data were analyzed from the Global Enteric Multicenter Study (GEMS) examining children <5 years old seeking care for moderate-to-severe diarrhea (MSD) in Siaya County, Kenya. MSD was defined as ≥3 loose stools in the previous 24 hours, with onset in the previous 7 days, and ≥1 of the following characteristics: loss of skin turgor, sunken eyes, dysentery, required IV rehydration or hospitalization. Stool specimens were tested for enteric pathogens, including by multiplex PCR for the gene targets of tEPEC (i.e. positive for both *eae* and *bfpA*). Demographic, clinical, and anthropometric data were collected at enrollment and at a ~60-day follow-up visit. To examine factors associated with tEPEC among MSD cases, a multivariable logistic regression model was constructed. Linear regression was used to assess linear growth faltering. Of the 1,778 cases enrolled between Jan 31, 2008 and Sep 30, 2012, 135 (7.6%) children tested positive for tEPEC. Among these, 85 (63%) had ≥1 additional enteric pathogen. Overall, 65% of tEPEC cases were infants 0-11 months old. There was a 3-fold greater odds of identifying tEPEC among infant MSD cases than among children in older age groups (adjusted odds ratio [aOR] 3.02, 95% CI: 1.73-5.24). The odds of tEPEC were higher for MSD cases with loss of skin turgor (aOR 2.86, 95% CI: 1.08-4.82) and convulsions (aOR 2.95, 95% CI: 1.17-7.45), compared to those without. Infant cases with tEPEC compared to those without were associated with linear growth faltering (p=0.002) between enrollment and follow-up. Among 36 infant MSD cases who died, 9 (25.0%) had tEPEC compared with 77 (10.8%) of 764 infants who survived (OR 2.98, 95% CI: 1.35-6.56). Typical EPEC was a significant contributor to morbidity and mortality among infants with MSD in rural Kenya. Interventions aimed at reducing the burden of tEPEC and its sequelae should be urgently investigated, prioritized and implemented.

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CAUSES AND CONSEQUENCES OF *GIARDIA* INFECTION IN THE FIRST TWO YEARS OF LIFE IN THE MAL-ED BIRTH COHORT

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Giardia is a common enteropathogen detected in both diarrheal and non-diarrheal stools among children in low-resource settings. We describe the epidemiology of *Giardia* in the first two years of life in MAL-ED, a multisite birth cohort study. From 2,089 children, 34,916 stools collected during monthly surveillance and diarrhea were tested for *Giardia* by enzyme immunoassay. We quantified the risk of *Giardia* acquisition, identified risk factors, and assessed the associations with micronutrients, gut biomarkers, diarrheal risk, and growth using multiple linear regression with general estimating equations. Incidence of at least one *Giardia* detection varied by site, from 37.7% in Brazil to 96.4% in Pakistan, and was more common in the second year of life. Exclusive breastfeeding (hazard ratio (HR) for first *Giardia* detection in monthly surveillance stools: 0.46, 95% CI: 0.28, 0.75), higher socioeconomic status (HR: 0.74, 95% CI: 0.56, 0.97) and recent metronidazole treatment (risk ratio for any surveillance stool detection: 0.69, 95% CI: 0.56, 0.84) were protective. Associations with hygiene and environmental risk factors suggest that the fecal-oral and waterborne routes may both be important modes of transmission. *Giardia* persistence (2+ consecutive detections) in the first 6 months of life was associated with reduced subsequent diarrheal rates in Pakistan, but not in any other site. *Giardia* was also associated with an increase of 0.25 (95% CI: 0.10, 0.40) in lactulose-mannitol ratio. Across sites, *Giardia* persistence in the first 6 months was associated with -0.29 z-score (95% CI: -0.53, -0.05) deficit in weight and -0.29 z-score (-0.64, 0.07) deficit in length. Because detection of *Giardia* in non-diarrheal stools was common, attributing diarrheal etiology to *Giardia* may often be inappropriate. However even in the absence of diarrhea, *Giardia* infection, especially when persistent in the first 6 months, may impact child development through stunted growth. Interventions to interrupt transmission may better reduce the burden and impact of *Giardia* than mass drug administration since metronidazole only transiently reduced detection.

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DETERMINANTS OF HEALTH AND PREVALENCE OF INFECTIOUS GASTROINTESTINAL DISEASE IN CHILDREN LIVING IN THE PERUVIAN AMAZON RIVER BASIN

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Diarrheal disease continues to be a leading cause of childhood morbidity and mortality in resource-poor settings, lacking proper water sanitation infrastructure. The community of Belen in Iquitos, Peru experiences intermittent seasonal flooding from the Ataya river, a tributary of the Amazon River. This project studied the differences in prevalence of various enteric pathogens as well as qualitative differences between "high" and "low" zones. Zone distinctions were based on levels of recent seasonal flooding, with low zones experiencing flooding throughout the majority of the year. Households involved in the study completed a questionnaire in Spanish and water was collected from the home's source and storage for

analysis of fecal coliforms and the presence of chlorine. Additionally, stool samples from a child under the age of five were obtained and screened for presence of bacteria and parasites. Samples were collected from 232 households. 100 households were determined to be in the low zone and the remaining 132 in the high zone. The overall prevalence of enteric pathogens in the high zone was 50% (66/132), compared to the 70% (70/100) prevalence in the low zone (p=0.0022). In both source and stored water, there was a higher rate of coliform contamination in the low zone (both p<0.0001). In addition, there was a difference in education levels between high and low zones (p<0.0001). Distinct educational differences between the high and low zones were observed in Belen that were associated with the presence of fecal coliforms in source and stored water and enteric pathogen prevalence. Overall, households in the low zone had lower education levels and experienced higher coliform contamination of their water and higher prevalence of enteric pathogens in stool samples. Additional studies or analysis will include sources of contamination of household water with respect to previously identified geographical differences.

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THE SHIFTING PATTERN OF *VIBRIO CHOLERAE* O1 SEROTYPES OVER A PERIOD FROM 1996 TO 2016 IN 2016 IN BANGLADESH

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Vibrio cholerae O1, the primary cause of epidemic cholera, has two biotypes, El Tor and Classical, and two major serotypes, Ogawa and Inaba. They have distinct phenotypes and differ with respect to the severity of the disease they can cause, the ability to survive outside the human host, and the seasonal pattern of infection. The changes of these serotypes with time and associated immune response in patients is important to understand the pathogenesis of cholera. We therefore followed the data for cholera for the last 20 years to determine the variability of the serotypes and its impact on demographic characteristics, clinical disease and on immune responses generated in cholera patients attending the diarrheal hospital at the icddr,b in Dhaka, Bangladesh. We analysed data from cholera patients at the icddr,b hospital from 1996 to 2016. Based on this data, we observed that the Ogawa serotype dominated during the period from 1996 to 1999 (80-99%), while the Inaba dominated from 2000 to 2002 (63-85%). From 2003 to 2007 we saw an alternative shift from Ogawa to Inaba (65% Ogawa, 74% Inaba,71% Ogawa, 60% Inaba, 52% Ogawa). However, in 2007 both serotypes were prevalent. However, following this cholera due to the Ogawa serotype started to increase and peaked in 2009-2010 (95-99%) and remained prevalent until December 2015 (89%). Interestingly, after 8 years of prominence of the Ogawa serotype, hospitalization of patients with *V. cholerae* O1 Inaba starts to increase in 2016 (January to March 2016) and reached a predominance of 92%. The cholera vaccine studies that we have been conducting in the urban slum of Dhaka city from the year 2011 to 2016 also enabled us to track this shift in 2016 to the *V. cholerae* O1 Inaba serotype. We found patients infected with Ogawa serotype were younger, presented with shorter duration of diarrhoea and frequent abdominal pain, vomiting and need for intravenous fluids though they had similar status of dehydration. We are also analyzing the immunological responses in the host as well as the genotypic and phenotypic characteristics of *V. cholerae* O1 strains isolated during this shift to the *V. cholerae* O1 Inaba serotype.

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EFFECT OF A BIVALENT, KILLED, WHOLE CELL ORAL CHOLERA VACCINE ON PREGNANCY OUTCOME IN BANGLADESH

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Evidence suggests that cholera during pregnancy has adverse effects on pregnancy outcome. A low cost oral cholera vaccine (OCV; Shanchol) appears to be an effective intervention to prevent cholera incidence. However, the vaccine is not recommended for pregnant women possibly due to lack of evidence of safety. In an efficacy study of a single-dose OCV in an urban slum of Dhaka city, some pregnant women took the vaccine unknowing of their pregnancy status. We therefore carried out this study to determine if the OCV is safe to administer during pregnancy. The objective of the study was to compare the differences in adverse pregnancy outcomes between the vaccine and placebo groups. A pregnancy screening visit among women of reproductive age (15-49 yrs) was conducted two months after the mass vaccination. We interviewed pregnant women whose pregnancy ended before the screening visit and collected necessary information related to their pregnancy outcome retrospectively. For women whose pregnancy was ongoing at the time of the screening visit, were followed up by visits until the pregnancy outcome occurred. The screening visit was conducted on 71,202 women of reproductive age and identified 1323 pregnancies: of these 550 were exposed to OCV during pregnancy and 773 exposed to OCV right before conception. Of 550 women, 405 had pregnancy outcome after the screening visit and were included in the primary analysis. We identified 7 (3.4%) adverse outcome from vaccine arm and 6 (3.0%) from placebo arm. OCV-exposure during pregnancy had no significant effect on adverse pregnancy outcome (Adj. OR: 1.11, 95% CI: 0.36, 3.53). We identified 7 (3.6%) preterm delivery from vaccine arm and 12 (6.1%) from placebo arm. We also found a non-significant protective effect of OCV on low birth weight. This study gave us no evidence of harmful effect on pregnancy outcome due to OCV exposure during pregnancy. The information from this study gives policy makers evidence to create awareness for offering OCV during pregnancy. This will be useful in reducing the number of cholera during pregnancy and also reduce adverse pregnancy outcome.

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ENHANCING DISTRICT-LEVEL CAPACITY TO INVESTIGATE AND RESPOND TO ACUTE DIARRHEAL DISEASE OUTBREAKS - TAMIL NADU, INDIA, 2013-2015

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Acute diarrheal diseases (ADD) account for 46% of reported outbreaks in India. Effective district-level response is constrained by lack of specimen collection, limited laboratory capacity, and incomplete epidemiologic investigation. In October 2013, Tamil Nadu introduced standard investigation tools and initiated focused training to strengthen ADD

outbreak response in two pilot districts, Cuddalore and Kanchipuram (population 2.6 million and 4.0 million, respectively). We reviewed investigation reports to characterize outbreak response over the two-year pilot period. We abstracted standard data for all ADD outbreak investigations conducted in both districts between November 2013-2015 to determine timeliness of response, proficiency in specimen collection and testing, completeness of epidemiologic investigation, and identification of outbreak source. Testing for *Salmonella*, *Shigella*, and *Vibrio* species was conducted at the district microbiology laboratory. In the two-year period, 17 ADD outbreaks were investigated in the two districts. District teams detected and responded to all outbreaks within 24 hours; patient specimens were collected in 13 (76%) outbreaks; bacterial etiology (*Vibrio cholerae* and *Shigella sonnei*) was confirmed in two (12%). Systematic case ascertainment was conducted in 13 (76%); an epidemic curve was developed in 8 (47%), and standard food-history questionnaires were utilized in 4 (29%). A case-control or cohort study was conducted in 5 (29%) investigations. A specific water (n=3) or food (n=7; including shellfish=2, fish=1, rice dishes=4) source was identified in 10 (59%) outbreaks. Over two years, pilot districts demonstrated the ability to lead timely and complete detection and response to ADD outbreaks. Systematic epidemiologic investigation methods supported the identification of food or water sources of illness in the majority of outbreaks. Focused efforts to strengthen district laboratory diagnostic and surveillance capacity will further enhance the ability to detect outbreak etiologies and contribute to global health security in India.

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INCREASING ANTI-ADHESIN IMMUNE RESPONSES BY MODIFYING FIMBRIAL GENE STEM-LOOP STRUCTURE IN LIVE ATTENUATED SHIGELLA/ETEC VACCINES

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Antibodies blocking the function of the adhesin protein of ETEC fimbriae are likely to be effective at preventing ETEC induced diarrheal disease. A way to increase the ratio of tip-adhesins to stem-proteins is desirable for live attenuated vaccines, not only to increase the amount of tip adhesin molecules presented to the vaccinee's immune system, but also to decrease the metabolic strain on the live attenuated vaccine candidate. Using RNA modeling in Geneious software we examined 15 ETEC operons of Chaperone-Usher (CU) type fimbriae and searched for genetic features controlling the production of tip and stem proteins. A stem-loop region was found to follow the stem-encoding genes in all fimbrial operons. We hypothesized that decreasing the stem-loop (SL) region would lead to decreased production of stem proteins, and therefore shorter fimbriae. Operon modification was performed by site-directed-mutagenesis in SL regions in CFA/I and CS5 operons cloned in plasmids and transformed into the live attenuated Shigella strain CVD 1208S. Clones CFA/I SLD1 and CS5 SLD6 with modified SL regions were examined with electron microscopy using negative staining. The lengths of fimbriae were measured in these and in non-modified clones using ImageJ open source software. Guinea pigs were immunized with the CVD 1208S-expressing CS5 SLD6 construct and serum and tears were analysed by ELISA for IgG and IgA responses the CS5 and Shigella LPS respectively. Clone CS5 SLD6 had mean fimbrial lengths of 96.5µm, while the wild type operon mean length was 306.7µm, $p<0.001$. Clone CFA/I SLD1 had a mean fimbrial length of 77.0 µm, while the wild type operon mean fimbrial length was 613.5 µm, $p<0.001$. In three guinea pigs immunised with CVD 1208S(CS5 SLD6) we found strong increases in IgG and IgA antibody towards both Shigella LPS and ETEC CS5. Modifying the stem-loop structure in ETEC fimbrial operons can be used to increase the tip-to stem protein ratio in live attenuated vaccines. The method is also likely to be applicable to other CU fimbriae. Further characterization of the CFA/I and CS5 short fimbriae live vaccine candidates are ongoing, and results will be presented.

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DIARRHEAGENIC *ESCHERICHIA COLI*: PREVALENCE AND PATHOTYPE DISTRIBUTION IN CHILDREN FROM PERUVIAN RURAL COMMUNITIES

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Diarrheagenic *Escherichia coli* (DEC) are common pathogens of childhood gastrointestinal infections worldwide. To date, research tracking DEC has mainly been completed in urban areas. This study aimed to determine the prevalence and pathotype distribution of DEC strains in children from rural Peruvian communities and to establish their association with malnutrition. In this prospective cohort, 93 children aged 6 to 13 months from rural communities of Urubamba (Andes) and Moyobamba (jungle) were followed for 6 months. Diarrheal and control stool samples were analyzed using Multiplex Real-Time Polymerase Chain Reaction (mRT-PCR) to identify the presence and virulence genes of DEC strains. A total of 820 specimens were collected, of which 46 (5.6%) were diarrheal and 774 (94.4%) were non-diarrheal. A median of 10 stool samples per child were collected. The overall isolation rate of DEC was 43.0% (352/820). Enteroaggregative (EAEC, 20.4%), Enteropathogenic (EPEC, 14.2%) and Diffusely aggregative *E. coli* (DAEC, 11.0%) were the most prevalent pathotypes. EAEC was more frequently found in Moyobamba samples ($p < 0.01$). EPEC was the only strain significantly more frequent in diarrheal than asymptomatic control samples ($p < 0.01$). DEC strains were more prevalent among younger children (aged 6-12 months, $p < 0.05$). A decline in Height-for-age Z-score (HAZ) was observed in 75.7% of children who completed follow-up (74/93). EAEC was more frequently isolated among children who had a greater HAZ decline ($p < 0.05$). Compared to periurban coastal areas, DEC strains were more frequently found in stool samples from children in rural communities of the highlands and jungle. Additionally, children with a greater decline in their growth rate had higher EAEC isolation rates, highlighting the importance of this pathogen in child malnutrition.

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BURDEN OF CHOLERA IN THE WHO EASTERN MEDITERRANEAN REGION (EMR): A MAPPING EXERCISE

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Cholera is a persistent public health burden in the WHO Eastern Mediterranean Region, but the geographical distribution of risk in the region is not well characterized. We conducted an assessment of the region's cholera risk in collaboration with the WHO. Cholera incidence data at the lowest available spatial and temporal level between 2005-2015 were obtained from Ministry of Health focal points in 8 countries: Afghanistan, Iran, Iraq, Pakistan, Somalia, Sudan, Syria, and Yemen. A literature review of cholera incidence was done to bolster data quality. For each country, we searched PubMed for "cholera" and the country name, restricted to January 1, 2005-October 31, 2015. A similar search of Index Medicus for the Eastern Mediterranean Region was conducted to retrieve regional publications not indexed internationally. A search of ProMED cholera reports for each country from January 1, 2005 was also conducted. Population data were obtained from the WorldPop project. Hierarchical models incorporating aggregated counts at different spatial scales were used to estimate cumulative incidence. Country-specific maps of 5-year cumulative incidence were created. Based upon the received cholera surveillance data, a total of 850,869 cases of acute watery diarrhea/suspected cholera were reported, including 3,037 confirmations and 2,679 deaths. There was notable variability in the quality, completeness and type of surveillance data received, both in terms of

geographical resolution (district level: Afghanistan, Pakistan, Sudan (2006 only), Iraq (2015 only), and Yemen; province level: Iran, Syria, Yemen; and national level: Somalia) and time period covered. Afghanistan, Pakistan and Somalia experienced seasonal outbreaks associated with the summer months. For countries with district-level data, outbreak severity varied, with the highest case fatalities observed in Sudan and Somalia. The results offer a preliminary characterization of cholera risk in 8 EMR countries. The identification of hotspots can inform targeted prevention and control efforts, including oral cholera vaccine use and water, sanitation and hygiene strategies.

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UNDERSTANDING HOW THE FEEDBACK BETWEEN DIARRHEAL DISEASE AND MALNUTRITION IMPACTS THE DYNAMICS OF ENTERIC PATHOGEN TRANSMISSION: A MATHEMATICAL MODELING APPROACH

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There is growing concern that environmental exposure to enteric pathogens influences long-term malnutrition-related morbidities- such as growth faltering, cognitive development, and chronic inflammation- among children under five in the developed world. Indeed, a dangerous feedback loop exists between infection and nutrition. This feedback-loop is influenced by interacting transmission pathways: nutritional status impacts susceptibility to infection, and diarrheal disease impacts absorption of nutrients to influence overall nutritional status. Thus, use of standard public health tools, such as regression analyses, to study this phenomenon violate assumptions of independence and provide little insight into the system. Rather, in order to appropriately assess the interconnectedness of infection and nutrition pathways a systems-based analysis is required. We use a stratified compartmental model, based on ordinary differential equations (ODE), to understand the mechanisms through which environmental-mediated enteric pathogens transmission is influenced by a child's nutritional status. This model accounts for subclinical and clinical infection states with diarrhea causing enteric pathogens stratified by malnourished and well-nourished children. Where appropriate, bidirectional transmission between nutritional states may occur. Model parameters were identified using enteric pathogen transmission rates as described in the literature. Through use of this model we present a holistic understanding of mechanisms by which environmental enteric dysfunction (EED) may be occurring at the community-level, highlighting potential opportunities for intervention.

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EXPLORING HUMAN GUT MICROBIOTA DIVERSITY ACROSS A RURAL TO URBAN GRADIENT IN ECUADOR AND THEIR RESPONSE TO DIARRHEAL INFECTIONS

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The gut microbiota plays a key role in health and prevention of invasion by pathogens. Environmental factors as well as host genetics and physiology shape the gut microbiota composition. Previous studies have reported that urban lifestyles impact the gut microbiota structure, revealing higher fecal bacterial diversity in less industrialized compared to industrialized settings. In this study, we sought to understand how geographical factors, including access to clean water and sanitation facilities, influence the signature of commensal gut microbiota in healthy individuals sampled as part of a case control study of diarrhea in four sites along a rural to urban gradient in Ecuador. Because diarrheal diseases are an important global health concern mainly in developing countries and little is known about the protective role of microbial diversity during diarrhea infection, we also evaluated the response of the gut microbiota during acute diarrheal disease (ADD). Preliminary taxonomic surveys based on 16S rRNA gene sequences in a group of 13 individuals living across a gradient of remoteness in Northern Ecuador showed that non-ADD (healthy) samples from individuals living in remote villages (rural areas) presented higher OTU richness than those living close to the main city. The estimated number of bacterial species in ADD samples was significantly decreased upon infection (paired t-test, $p = 0.01$). Based on these promising results, we are currently profiling the gut microbiota of 120 fecal samples obtained from 60 individuals living along a more extensive urban-rural gradient and we will report on the results of this analysis. This study combines the effects of enteric infection and geographical factors on the gut microbiome into the same analysis to gain a better understanding on how resilience and geography modulate the gut microbial response during an acute infection.

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THE USE OF ZINC FOR TREATMENT OF CHILDHOOD DIARRHEA IN RURAL WESTERN KENYA, 2010-2014

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Diarrhea is a leading cause of morbidity and mortality among children globally. The World Health Organization Integrated Management of Childhood Illness recommends zinc and oral rehydration solution (ORS) for treatment of all childhood diarrhea irrespective of concurrent symptoms or diagnoses. ORS has been used for decades while the recommendation for zinc in Kenya was implemented in March 2010. Limited data are available on the uptake of zinc for diarrhea management. From June 2010 to May 2014 we examined factors associated with use of zinc among children aged <5 years with medically-attended diarrhea within a population-based infectious disease surveillance platform in rural western Kenya (population <5 years ~4,200). Participants received free care at a study clinic where history of illness, signs/symptoms, diagnosis and management were captured in a structured questionnaire. Diarrhea was defined as ≥3 loose stools in 24 hours. Overall 1,561 cases of diarrhea among 1,123 children were managed at the clinic; 870 (56%) cases were treated with both zinc and ORS, 44 (3%) cases received zinc without ORS, and 657 (41%) cases received no zinc. Factors positively associated with use of zinc included a history of vomiting everything (OR 1.4, 95% CI 1.1-1.8), sunken eyes (OR 2.3, 95% CI 1.4-3.9), unable to feed/ breastfeed (OR 2.6, 95% CI 1.2-5.6) and treatment with ORS (OR 19.7, 95% CI 13.6-28.5). Children aged ≥24 months were less likely to receive zinc compared to children aged <24 months (odds ratio [OR] 0.6, 95% confidence interval [CI] 0.5-0.8). Other factors negatively associated included mucus in diarrhea (OR 0.7, 95%CI 0.6-0.9), malaria diagnosis (OR 0.5, 95%CI 0.3-0.9), treatment with antibiotics (OR 0.3, 95%CI 0.2-0.4) and anti-malarials (OR 0.5, 95%CI 0.3-0.9). Overall the use of zinc for diarrhea management was low. Young children at risk for dehydration are more likely to receive zinc. However clinicians seem to be underutilizing zinc for older children and those with a concurrent clinical diagnosis such as malaria. Further training of health care workers on recommendations for zinc use is needed.

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DECREASED VIRULENCE ASSOCIATED WITH INCREASE EARLY PRO-INFLAMMATORY CYTOKINE INDUCTION BY MYCOBACTERIUM AFRICANUM INFECTED IN MATURE HUMAN MONOCYTE-DERIVED MACROPHAGES

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Mycobacterium africanum (MAF) having been sub-divided into MAF West African 1 (MAF1) (Lineage 5) and MAF West African 2 (MAF2) (Lineage 6), are 2 distinct phylogenetical lineages within Mycobacterium tuberculosis complex (MTBC) and together with Mycobacterium tuberculosis *sensu stricto* (MTBss) (Lineage 4) are the cause of human tuberculosis (TB) in West Africa. However within central West Africa, Ghana represent one of the few countries (Sierra Leone, Ivory Coast and Benin) that are known to have both distinct phylogenetic lineages of MAF. Thus there is little or no knowledge available as to whether their genetic diversity may have a significant variation in terms of virulence in a host-pathogen interaction compared to MTBss.Strains of Lineage 5 and Lineage 6 circulating in South-Western Ghana as well as a comparator Lineage 4 (Cameroon sub-lineage) were identified. We assessed two virulence associated characteristics: mycobacterial growth in mature human monocyte-derived macrophages (MDM) and early pro-inflammatory cytokine induction from 4hrs to 72hrs.In MDM, Lineage 5 strains grew significantly slower than Lineage 4 strains from 24hrs to 72hrs (p&lt0.05). Similarly Lineage 6 strains also grew significantly slower than Lineage 4 strains over the same time period (p&lt0.05). In contrast the mean doubling time of Lineage 5 strains was significantly higher than Lineage 4 strains (p&lt0.05) at 72hrs. Likewise Lineage 6 strains were significantly higher than Lineage 4 strains (p&lt0.05). Lineage 5 strains induced higher levels of early pro-inflammatory cytokines (TNF-α, IL-6 and IL-12p70) than Lineage 4 strains from 24hrs to 72hrs (p&lt0.05). Similarly Lineage 6 strains also induced higher levels of pro-inflammatory cytokines than Lineage 4 strains over the same time period (p&lt0.05).The data shows MAF had a low intracellular growth rate and a higher doubling time in MDM. Likewise MAF induced hyper-inflammatory response thereby inducing a 'slow growth' phenotype highlighting the point that MAF indeed has lower virulence and longer latency leading to slower progression to active disease in the host.

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ANTIBIOTIC RESISTANCE PATTERNS OF COMMON GRAM-NEGATIVE UROPATHOGENS IN ST. PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE, ETHIOPIA

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The resistance of bacteria causing urinary tract infection (UTI) to commonly prescribed antibiotics is increasing both in developing as well as in developed countries. Resistance has emerged even to more potent antimicrobial agents. The study was undertaken to report the last three year antibiotic resistance pattern among common bacterial uropathogens in St.paul's Hospital Millennium Medical College. A total of 2544 urine samples were processed in the last three years starting from September 2012 to 2015. Inoculation was performed onto blood agar and MacConkey agar symoultaniously. Significant bacteriuia was considered with colony counts greater than 105cfu/ml, for a single isolated bacterium. Isolated organisms were identified by conventional biochemical methods. Antibiotic susceptibility was done by Kirby Bauer disk diffusion method. Data entry and analysis was done using SPSSv20. Of the total 2544 samples, 569(22.4%) showed significant growth. Gram negative organisms totaled 508(20.0%), and 61(2.4%) isolates were gram positive. The most frequently isolated gram negative bacterium was *E. coli* followed by *Proteus* and *Klebsiella* spp. 305 (12.5%), 102(4.0%), and 42(1.7%) respectively. Between 2012 and 2013, the resistance rate to Tetracyclin, Ampicilin, Amoxycilin, and Nalidixic Acid was reported as 69%, 64% and 67% respectively. In 2014, the study showed that resistance pattern for all except to tetracycline becomes increased 65%, 78% and 78%. In 2015, however, there was no a single organism which is sensitive to the above listed drugs. The study also showed an emerging resistance to Ciprofloxacilin and Ceftriaxone especially for common gram-negative bacteria. There was relatively low resistance rate to Nitrofurantoin, Gentamycin, and Trimethoprim-Sulfamethoxazole throughout the years. In conclusion, in this study setting resistant rate to Tetracyclin, Ampicilin, Amoxycilin, and Nalidixic Acid were high. Increasing antibiotic resistance trends indicate that it is imperative to rationalize the use of antimicrobials in the community and also use these conservatively.

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FIVE-YEAR SURVEILLANCE OF DIPHTHERIA OUTBREAK IN INDONESIA

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Diphtheria outbreak has become a major problem in Indonesia since 2011. East Java province in Java Island is the most severely affected area. The objective of this study is to analyze the five-year (2011-2015) surveillance report of diphtheria outbreak in East Java Indonesia. This study was based on surveillance data collected at East Java Provincial Health Office from all districts since January 2011 until December 2015. The data came from the district and provincial hospitals, the local health officers, the family of the patients, and the contacts. Microbiology data were collected from one international standard diphtheria laboratory in Surabaya. Here are the results. For five years period since 2011, there were 3004 cases reported from 38 districts (100%), with the peak at 2012 (955 cases). Based on WHO data, this number was the second rank in the world after India. The case fatality rate was 3.4% (103 patients). Male (1607, 53.4%) slightly outnumbered female. Although most patients were below 15 years old (2111, 70.2%), the trend showed the increasing proportion of adults. In 2012, based on the immunization status, the percentage of unimmunized patients, partially immunized, and completely immunized by age were 39%, 49.3%, and 11.7%, respectively. Among those deceased, the youngest and oldest age were 11 month and 70 years, respectively. Only 187 nasal and throat swab specimen were positive for toxigenic *Corynebacterium diphtheriae*. Despite many efforts such as multiple outbreak response immunization (ORI) especially in 2011-2013 this outbreak could not be stopped. As the conclusion, for five years since 2011 there was a diphtheria outbreak in East Java Indonesia. The highest number of patient was in 2012. Most of the patients were not completely immunized. The positivity rate of microbiology culture was low. Many actions in affected area has not been enough to stop the problem.

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TRENDS OF SEVERITY AND OUTCOMES OF PATIENTS WITH PNEUMOCOCCAL PNEUMONIA IN RELATION TO SEASONALITY IN NORTHERN AND SOUTHERN HEMISPHERES

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Despite efficacious antibiotics and vaccines, pneumococcal pneumonia remains a major cause of morbidity and mortality. Incidence of invasive

pneumococcal disease demonstrates a distinct seasonal course in temperate climates, with infection incidence peaking in winter months, the forces which drive this seasonality remain poorly understood. Understanding the trends of invasive pneumococcal pneumonia can aid prevention of pneumococcal pneumonia mortality, which has been reported as upwards to 60% in susceptible populations. We investigated the trends and outcomes of patients with invasive pneumococcal pneumonia in relation to seasonality in the northern and southern hemispheres using data from the Community Acquired Pneumonia Organization (CAPO) databas, an international database of confirmed community-acquired pneumonia cases. Cases of blood cultures positive Streptococcus pneumoniae from multiple sites from both northern and southern hemispheres were included in the anyalsis. Cases from sites with tropical climates were excluded. Prevalence by season was analyzed by chi square testing. Mortality by season was analyzed by multivariable logistic regression, adjusting for pneumonia severity index score, ICU admission, history of chronic obstructive pulmonary disease, and pneumococcal bacteremia. Time to clinical stability and length of hospital stay were analyzed using Kaplan Meier survival curves. Of 4,507 cases of pneumococcal pneumonia, 425 cases met the inclusion criteria. Winter, spring, summer, and fall accounted for 36%, 29%, 9%, and 26% of the cases, respectively. There was a significant decrease in the incidence of invasive pneumococcal pneumonia during the summer ($p<0.001$). Of the 425 cases, 317 (75%) occurred in the northern hemispheres and 108 (25%) in the southern hemisphere. There was no significant difference in mortality, time to clinical stability, or length of hospital stay between these two groups. We found that incidence of pneumococcal pneumonia exhibited a marked seasonality in both hemispheres. However, we found no association between clinical outcome and season of the year.

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MOLECULAR-BASED ASSAYS REVEAL *STREPTOCOCCUS PNEUMONIAE* AS THE LEAD ETIOLOGICAL AGENT IN THE ONGOING MENINGITIS EPIDEMIC IN GHANA

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Like most other countries within the meningitis belt, Ghana experiences sporadic outbreaks of bacterial meningitis frequently; however, the current epidemic in Ghana has received significant impact and media attention. In response to the current bacterial meningitis epidemic in Ghana, rapid diagnostic test kits such as Pastorex, a family of rapid, latex agglutination test and Gram stain are used for detecting meningitis within affected health facilities. However, these tests fail to identify the serotype of the bacteria causing the infection, which is critical to initiating population-level interventions such as vaccination. We therefore aimed to use molecular techniques to aid in the characterization of suspected agents responsible for the current epidemic of meningitis in Ghana. To determine the prevalence and etiology of meningitis, we investigated cerebrospinal fluid (CSF) specimen from 161 individuals suspected of meningitis using standard microbiological methods and a Fast Track Diagnostics (FTD, Luxemburg) real time multiplex polymerase chain reaction (PCR) assay. This multiplex PCR assay consists of primer/probe mix and allows simultaneous detection of N. meningitidis, S. pneumoniae and H. influenzae. In all, 93.3% (148/161) of the cases were from the Brong-Ahafo region, while the remaining were from Greater Accra and Ashanti regions. In total, 53% (85/161) were female with median age of 21 (0.3 - 83 years) for both sexes. A total, 48% (77/161) of the patients were positive for bacterial meningitis; 73% (56/77) were *Streptococcus pneumoniae*, 26% (20/77) were *Neisseria meningitidis,* while 1% (1/77) was positive for *Haemophilus influenzae*. Interestingly, 2.6% (2/77) patients were co-infected with both S. pneumoniae and *N. meningitidis*. PCR-based assay implicates S. pneumoniae as the principal etiologic agent followed by *N. meningitidis* in the ongoing meningitis epidemic in Ghana. In addition to providing necessary logistics and other interventional measures, we highly recommend existing research institutions and referral hospitals within the affected regions should be equipped with molecular based-capacities.

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THE MOLECULAR EPIDEMIOLOGY OF *STAPHYLOCOCCUS AUREUS* SKIN AND SOFT TISSUE INFECTIONS IN THE LAO PEOPLE'S DEMOCRATIC REPUBLIC

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This is the first report of the molecular epidemiology of *Staphylococcus aureus* from skin and soft tissue infections (SSTI) in Laos. Neighbouring countries report high MRSA rates but these are rare in Laos. We selected a randomized sample of 96 *S. aureus* isolates from SSTI samples received at Mahosot Hospital Microbiology Laboratory, Vientiane, between July 2012 - June 2014, including representation from 7 referral sites. All isolates underwent susceptibility testing by CLSI methods, *spa* typing and DNA microarray analysis (Alere Technologies). Whole genome sequencing was performed for rarely described lineages. 43 *spa* types, representing 17 different lineages, were identified. Median age was 19.5 years (IQR 2-48.5 years); 52% were female. The dominant lineage was CC121 (n=39; 41%): all but one encoded Panton-Valentine leukocidin (PVL) and 49% (n=19) were recovered from children aged <5 years. 58% of all isolates (n=56) encoded PVL (representing 6 lineages); half of these (28/56) had abscesses; 3 had positive blood cultures. *S. argenteus* (part of the *S. aureus*-related complex) was identified in 6 (6%) cases; mostly adults >50 years and diabetics. 6 isolates (6%) belonged to a rare lineage, ST2885, with 3 possibly associated with cross-infection in a paediatric intensive care unit. One previously undescribed strain was identified (sequence type pending). Resistance to antibiotics was uncommon, except for penicillin (93; 97%), tetracycline (48; 50%) and fosfomycin (64; 67%) predominantly encoded by *blaZ*, *tet(K)* and *fosb* respectively. 7 (7%) MRSAs were identified, belonging to ST239-MRSA-III, CC59-MRSA-V(T) Taiwan Clone, ST2250-MRSA-IV, ST2885-MRSA-V and CC398-MRSA-V: 3 patients had had recent healthcare contact and 1 had recently travelled. Globally widespread CC5 and CC30 were absent. Our report shows parallels between Laos and neighbouring countries, highlights the prominence of PVL in SSTI and suggests infiltration of MRSA clones of epidemic potential from surrounding countries. Continued vigilance is warranted considering the paucity of antibiotic options in Laos and challenges for robust infection prevention and control.

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DISTINCT ARRANGEMENTS OF VIRULENCE GENE EXPRESSION IN UROPATHOGENIC *ESCHERICHIA COLI* STRAINS

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Most urinary tract infections (UTIs) are caused by uropathogenic *Escherichia coli* (UPEC). The aim of this study was to determine the patterns of expression of genes coding for iron uptake *(iuc, iroN* and *irp2*), adhesins (*fim, afa1, sfa, iha, tsh, papGI, papGII* and *papGIII*), protectins (*KpsMT, ompT* and *iss*) and toxins (*cnf1, hlyA*, *set-1, astA, vat, usp* and *cva/cvi)* in UPEC strains. Using standard biochemical tests, followed by PCR amplification of 16S rRNA gene, we identified 194 strains as *E. coli,*

which were isolated from patients with UTIs at Unidad Médica Familiar No. 64 (Instituto Mexicano del Seguro Social), Estado de México. Virulence gene expression in UPEC strains was determined by real-time PCR after infection of *in vitro* cultured A431 human vaginal cells. Sixty-eight percent (n=132) of UPEC strains expressed *iuc* gene*;* 65% (n=126) *iha*; 61.3% (n=119) *KpsMT;* 59.2% (n= 113) *fim*; 48.4% (n=94) *irp2;* 31.4% (n=61) *set-1;* 31% (n=60) *astA;* 15.5% (n=30) *papGII;* 12.3% (n=24) *afaI*; 11.8% (n=23) *hlyA*; 10.3% (n=20) *iroN;* 9.8% (n=19) *ompT;* 5.7% (n=11) *papGIII* and *vat*, in each case*;* 4.1% (n=8) *papGI* and *iss*, in each case; 3.1% (n=6) *iuc;* 2.6% (n=5) *sfa* and *tsh*, in each case; 1.5% (n= 3) *cva/cvi* and 0% *cnf1.* A total of 106 distinct arrangements of virulence gene expression were identified in the UPEC strains. The most abundant of them (*irp2/ fim/iha/kpsMT/usp*: iron acquisition system/ adhesins/protectin/toxin) was represented by 28 strains (14.1%). Findings of this study revealed that combinations of virulence genes are expressed during infection of A431 cells with UPEC strains, showing that these strains could be highly virulent and cause more severe infections as cystitis or pielonephritis.

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METHICILLIN-SENSITIVE AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) CARRIAGE AT A UGANDAN REGIONAL REFERRAL HOSPITAL

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Despite increasing antimicrobial resistance globally, data are lacking on the prevalence and associated risk factors for *Staphylococcus aureus* (SA) and methicillin-resistant SA (MRSA) carriage in low-resource settings. We enrolled a cross-sectional sample of 500 Ugandan adults from a predominantly rural agricultural area seeking care at a regional referral hospital or clinic, swabbed anterior nares, and tested for SA and MRSA carriage using Cepheid Xpert SA Nasal Complete assay. Mean age was 37 years and 322 (65%) were female. Of 498 participants reporting clinical data, 368 (74%) were currently taking antibiotics, 118 (24%) were recently hospitalized, and 315 (63%) had known risk factors for SA infection including open wounds (188; 38%), rash (99; 20%), recent immunosuppression (66; 13%), or HIV infection (166; 33%). Of 499 samples with a valid Xpert result, 145 (29%) were SA positive and 14 (2.8%) were MRSA positive. SA carriers were more likely than SA non-carriers to be male (44 *vs.* 32%, *P*=0.008) or have a chronic disease (61 *vs.* 47%, *P*=0.005), but less likely to report recent β-lactam antibiotic use (63 *vs.* 73%, *P*=0.02) or open wounds (32 *vs.* 40%, *P*=0.07). SA carriage ranged from 19% on maternity ward to 36% on medical ward (*P*=0.04). Though cases were few, MRSA carriers did not differ from non-carriers by sex (50 *vs.* 35% male, *P*=0.25) or chronic disease status (71 *vs.* 50%, *P*=0.17), but were more likely to be inpatients (86 *vs.* 59%, *P*=0.05), have an open wound (71 *vs.* 37%, *P*=0.01) have contact with pigs (21 *vs.* 5%, *P*=0.04); and less likely to report recent β-lactam use (43 *vs.* 71%, *P*=0.02). MRSA carriage ranged from 0% in HIV clinic to 8% on surgical ward (*P*=0.01). Using multivariable logistic regression, independent predictors of SA carriage were chronic illness (OR 1.73, *P*=0.007) and female sex (OR 1.69, *P*=0.01). β-lactam use was protective (OR 0.6, *P*=0.02). In Uganda, we found low overall prevalence of MRSA carriage, a protective association between β-lactam use and SA and MRSA carriage, and no association between carriage and HIV status. Further research should explore possible increased prevalence of MRSA carriage among hospitalized surgical patients.

1050

DEFINING THE MECHANISMS OF PROTECTIVE IMMUNITY ELICITED BY TWO VACCINE CANDIDATES AGAINST *ORIENTIA TSUTSUGAMUSHI* INFECTION

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Scrub typhus is an acute, febrile and potentially fatal disease and caused by infection with the obligate intracellular bacterium, *Orientia tsutsugamushi*. It is the most common rickettsial disease seen in the Asia-Pacific region. Since there is no vaccine available, creation of a safe and effective vaccine remains an important goal for public health. Recombinant antigens r56kp and r47kp have been shown to provide up to 100% protection in a mouse challenge model with two different strains. However, the mechanisms of protective immunity against *O. tsutsugamushi* challenge induced by these antigens remain unclear. To determine whether r56kp or r47kp subunit vaccine-induced protection depends on antibody- or T cell-mediated protective immunity in mouse models, we investigated 1) if adoptive transfer of either immune sera or T cells from r56kp- or r47kp-vaccinated mice would provide protection against *O. tsutsugamushi* challenge in naive recipient C3HeB/FeJ mice; 2) whether depletion of CD4+ T cells or CD8+ T cells would affect the ability of r56kp and r47kp vaccine to confer protection against *O. tsutsugamushi* challenge in C3HeB/FeJ mice; and 3) if B cell, T cell, CD4+ T cell or CD8+ T cell deficiency in mice would significantly affect the ability of r56kp and r47kp vaccine to confer protection against *O. tsutsugamushi* challenge. Our results suggest that i) both B cells and T cells contributed to r56kp and r47kp vaccine induced protection; ii) antibody and B cells may play a critical role in r56kp vaccine-induced protection; and iii) T cells may be more crucial for r47kp vaccine-induced protection.

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SCRUB TYPHUS: A LONG NEGLECTED PUBLIC HEALTH THREAT IN THE ASIA-PACIFIC AREA AND WORLDWIDE

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Scrub typhus is a serious public health problem in the Asia-Pacific area. It threatens one billion people globally, and causes illness in one million people each year. It can cause severe multiorgan failure with a case-fatality rate up to 30% without appropriate treatment. The antigenic heterogeneity of *Orientia tsutsugamushi* results in reinfection with scrub typhus. As a neglected disease, there is still a large gap in our knowledge of the disease, as evidenced by the sporadic epidemiologic data and other related public health issues regarding scrub typhus in its endemic areas. Our objective is to provide a systematic analysis of current epidemiology, diagnosis, treatment, prevention and control of scrub typhus in its endemic area and the rest of the world. Preliminary studies have demonstrated the wide and long existence of this endemic disease. We analyzed the epidemiology of scrub typhus through a thorough review of the epidemiology and public health impact of the disease. This study leads us to understand the disease, and provides a foundation for prevention and control. We then described the diagnosis and treatment of scrub typhus, which facilitates the development of the next generation of diagnostics and treatment. Atypical flu-like symptom and repeated failure to respond to antibiotics make the diagnosis and treatment considerably challenging. The last part of the project focuses on the prevention and control of scrub typhus in the Asia-Pacific region and worldwide. The antigenic diversity impedes vaccine development for the prevention of the disease. Our laboratory has been working on the interactions between host immunity and the pathogen. This analysis could largely benefit the control and

prevention of scrub typhus. The research also provides foundations for exploring new measures for other infectious diseases and public health threats.

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PERSISTENCE OF ST217 CLONAL COMPLEX LINEAGE B OF SEROTYPE 1 PNEUMOCOCCAL MENINGITIS IN NORTHERN GHANA - THE PROSPECT OF PNEUMOCOCCAL VACCINES IN AFRICAN MENINGITIS BELT

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Sporadic outbreaks of Serotype 1 pneumococcal meningitis were observed in Northern Ghana between 1998 and 2005 period. To monitor the molecular epidemiology of this strain, a hospital case- based surveillance was maintained from 2006 to 2011 period. Case and Laboratory based surveillances on pneumococcal meningitis were conducted. Specimens collected were analyzed using classical and molecular microbiology. The incidence rate was 18/100,000/population-year. Since the detection of ST217 clonal complex lineage B of serotype 1 pneumococci in this region, this clone persisted for 13 years as the main aetiology. The risk population shifted from the very younger children to adult age group. The host age distribution pattern was more associated with the biology of the strain than the exposure history of the study population. In conclusion, the incidence and case-fatality rates declined by 3 and 7 percentage points, respectively Nevertheless, the changes in age distribution pattern has major policy, programmatic and research implications. The immunization strategy ought to be refined to cover the new at risk population. Strategic platform ought to be developed to coordinate research outcomes of other allied scientist in Ghana to manage pneumococcal infection comprehensibly. Sero-surveillance should be conducted to evaluate the impact of PCV13 introduced in Ghana in 2012. Further research will be needed to consolidate our understanding on the association between strains' biology and host-age trends.

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PREVALENCE OF MATERNAL CARRIAGE OF GROUP B STREPTOCOCCUS AND *ESCHERICHIA COLI IN* SOUTHERN MOZAMBIQUE

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Group B streptococcus (GBS) and *Escherichia coli* are leading causes of neonatal sepsis in many industrialized countries. Reports from low-income countries infrequently identify those pathogens among newborns with sepsis. We assessed the prevalence of GBS and E.coli colonization among pregnant women in a rural Mozambican hospital. A cross sectional study was conducted on pregnant women attending Manhiça District Hospital at two different time-points during their pregnancy (1: during routine antenatal clinics (AC) at gestational age >34 weeks; 2: at delivery, regardless gestational age). Samples from lower genital tract and rectum for GBS and a vaginal sample and urine for E.coli determination were cultured. Thirty-six of the 200 pregnant mothers recruited at the AC (18%) were GBS carriers. Twenty-five of them (12.5%) had positive E.coli culture in their vaginal samples and 4/200 (2.0%) in the urine cultures for E.coli. One hundred and twenty mothers were recruited at delivery. Prevalence of GBS carriers in this group was 26.7% (32/120) and 22.5% (27/120) had positive E.coli culture in vaginal samples and 5% (6/120) in urine. Colonization by *E. coli* vaginal was significantly more common in women recruited at delivery (OR: 2.0, 95% CI 1.1-3-7). HIV-positive status was positive for 117/320 women (36.6%). There was no association between being colonized by GBS and HIV-positive status or others maternal risk factors. Almost 10% of the GBS isolates were resistant to penicillin (5.8% intermediate resistance and 3.8% fully resistant), the usual antibiotic utilized in the developed world to prevent GBS vertical transmission. All GBS isolates except three (2.9%) were sensitive to ampicillin, two of which were highly resistant to both ampicillin and penicillin. We will present results of serotyping and maternal antibodies. The study showed that GBS colonization among near term pregnant mothers is reasonably high in our community. Since screening and intrapartum antibiotic prophylaxis is difficult to implement in low-income countries, effort to prevent GBS vertical transmission should lead to provide effective vaccines.

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QUANTIFYING THE INDIRECT EFFECTS OF *HAEMOPHILUS INFLUENZAE* TYPE B VACCINATION IN CHILDREN UNDER AGE 5 YEARS

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Haemophilus influenzae type b (Hib) was a significant cause of morbidity and mortality among children under 5 years before an effective vaccine became available. Hib vaccine reduced disease beyond that expected from direct protection alone, indicating indirect effects in those not vaccinated provide meaningful protection. We modeled the magnitude of indirect effects as a function of time and population-level vaccine coverage. We regressed the observed disease reduction from pre-post vaccine introduction on the proportion vaccinated among children <5 years. We also compared the observed disease reduction to the expected disease reduction (calculated using the <5 age distribution of invasive Hib disease before vaccine introduction and vaccine rollout schedule) due to direct effects alone to estimate an indirect effect multiplier that can be used to calculate vaccine "effective coverage" to more completely estimate the proportion of the population protected against disease. We validated results by comparing to a study that empirically measured indirect effects on individuals. Using 23 data points identified from 10 studies, the model estimated that 40% vaccine coverage results in 39% (95%CI: 23%, 55%) additional children protected by indirect effects (i.e., 79% effective coverage), the highest indirect effect multiplier (x1.39) estimated; with 60% vaccine coverage over 85% (95%CI: 69%, 100%) disease reduction was predicted. The magnitude of the indirect effect reduces as vaccine coverage increases. Modeled results were similar to those measured empirically. In conclusion, the indirect effects of Hib vaccine in unvaccinated children have substantial impact on disease and in conditions of moderate (e.g., 40%) vaccine coverage can almost equal direct effects. These results can be used to improve estimates of expected disease reduction due to vaccines and should be incorporated into cost-effectiveness analyses. This approach can be applied to other vaccines, such as pneumococcal conjugate vaccine, which may impact vaccination policy decisions regarding introduction.

1055

BACTERIAL VAGINOSIS IN DAKAR (SENEGAL) IN 2015

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To point-out the importance of bacterial vaginosis (BV) in genital infections and to determine epidemiological, clinical and paraclinical factors linked to BV in Dakar. 1509 females patients enrolled in this study were interviewed

prior to the vaginal fluid collections. Prior to microscopic examination, both genital area and fluids were observed macroscopically. Cultures were performed for bacterial and fungal microorganisms. BV diagnosis was based on the presence of clue cells, pH > 4.5, and absence of Lactobacilli. Statistical analysis was done by Khi2 test and the Odds ratio. Among 1509 females, 33.5% had BV, 21% had Candida, 2.3% Trichomonas vaginalis infection and only 0.4% (N=6) were with gonorrhea. We found more BV from females aged from 25-34 than those aged from 35-44. But Odds ratio shows that BV is not linked to any age. BV is more found in married women than in celibate. In most of cases, genital area was normal. 35.7% cervix were found in association with BV. 39.8% of inflammatory reaction were linked to BV. In 23.1% of BV *Gardnerella vaginalis* was associated with Mobilluncus. Vagina flora was type III or IV in 99.2% of cases. In Dakar, BV belongs to the major genital infections and its management must be effective in Gynecologic and Obstetric services and also in National Campaign against AIDS since it eases the transmission of HIV.

1056

CHANGE IN NASAL COLONIZATION WITH *STAPHYLOCOCCUS AUREUS* IN ACTIVE PERUVIAN MILITARY POPULATION AFTER 1-YEAR FOLLOW-UP

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Antibiotic resistance is one of the greatest threats to the global health. Methicillin-resistant *Staphylococcus aureus* (MRSA) is distributed worldwide and asymptomatic carriers can be a source of transmission. Military personnel are exposed to some characteristics such as being in close quarters, less opportunities for hygiene during training and combat operations, etc., that make them susceptible to being colonized by *S. aureus*, and increasing the risk of infections by *S. aureus* as it occurs in military trainees. We conducted a prospective cohort study at the four largest bases of the Peruvian Air Force, collecting nasal swabs from 756 active duty military personnel (655 at baseline, and 101 at 6 month visit) during 1-year. The goal was to assess the rates of nasal colonization with *S. aureus* at baseline, after 6 months and 1-year follow-up. The samples were cultured to identify the presence of *S. aureus*, and the antimicrobial resistance profile was assessed via disk diffusion. We analyzed the change in the nasal colonization status among those participants who provided at least 2 samples. Only 484 participants met this requirement (390 after 6 months and 94 after 1 year). Nasal colonization with *S. aureus* was lower at baseline (9.7%, n=73/655), but increased over the study period up to 20.4% (n=70/343) among those who supplied a sample at 1-year visit. The incidence rate of nasal colonization (those who changed from negative to positive status) was 11.2%, while the percent clearance was 51%. Two participants were colonized with a MRSA strain (USA 300, SCCmec type IV) after 6 months of follow-up. At 12 months we did not isolate MRSA strains among our participants. The risk of being colonized with *S. aureus* among those participants with a first negative sample, increased in those with a diagnosis of skin and soft tissue infections or who were mobilized to different geographic areas due to military missions but none of them was statistically significant. This was the first study to systematically determine the prevalence and the molecular characteristics of MRSA among Peruvian active duty military population in multiple cities in the country.

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BLOCKADE OF CTLA-4 IMPAIRS ANTIBACTERIAL IMMUNITY BY REDUCING ACTIVATION OF CD8+ T CELLS AND INCREASING PRODUCTION OF IL-10 BY T CELLS IN MURINE *ORIENTIA TSUTSUGAMUSHI* INFECTION

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Post-acute persistence is a hallmark of *Orientia tsutsugamushi* infection in humans and rodents. We hypothesized that negative T cell co-stimulation plays a role in reducing the effector response to *O. tsutsugamushi*, thereby facilitating pathogen persistence. We had shown before that highest bacterial loads are found in the lung in our C57BL/6 mouse model of intradermal *O. tsutsugamushi* infection. Thus, we studied the role of negative costimulation and particularly CTLA-4 in pulmonary T lymphocytes. During the acute phase of infection, a significant reduction in CD4+foxp3+CTLA-4+ regulatory T cells was observed in the lung. However, on CD4+ and CD8+ T cells, expression of CTLA-4, PD-1, TIM-3 and LAG3 increased significantly during acute infection. In order to study the functional significance of CTLA-4 in this model, we blocked CTLA-4 using a monoclonal antibody before and during infection, and analyzed its influence on the pulmonary immune response and bacterial clearance. Other than expected, CTLA-4 blockade had profound effects on composition and phenotype of the pulmonary lymphocyte compartment: (1) more CD4+ and less CD8+ T cells invaded the lung, (2) expression of the activation markers CD44, KLRG1 and CD11a on CD8+ T cells was significantly reduced, and (3) PD-1 expression was increased. Moreover, CTLA-4 blockade caused a significant increase of bacterial loads in the lung. This was not due to decreased production of cytokines, since neither the ability of CD4+ nor CD8+ pulmonary T cells to produce IFN-gamma and IL-2 was impaired. However, re-stimulated splenocytes from mice receiving anti-CTLA-4 treatment revealed an increased production of IL-10. Thus, unlike in other infection models, our data suggest that CTLA-4 blockade is unable to increase effector responses in *O. tsutsugamushi* infection. Instead, its blockade reduces activation of CD8+ T cells and increases production of the regulatory cytokine IL-10, pointing to a novel role of CTLA-4 in *O. tsutsugamushi* infection.

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ANTIBIOTIC RESISTANCE PATTERNS IN BACTEREMIC CHILDREN FROM HOLOENDEMIC *PLASMODIUM FALCIPARUM* MALARIA REGION OF WESTERN KENYA

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Bloodstream bacteria commonly complicate malaria and other illnesses in children residing in *Plasmodium falciparum* holoendemic areas of sub-Saharan Africa. The emergence of antibiotic resistance by blood-borne bacteria increasingly complicates the clinical management of sick children. However, the paucity of data on the prevalence and patterns of antimicrobial resistance of bloodstream isolates continues to hinder rationale management and antibiotic use in pediatric populations. This study investigates antibiotic resistance patterns in bacteremic children (N=158) with malaria [+] (n=90) and without malaria [-] [n=50] upon enrollment at the hospital and during subsequent acute illnesses [n=18]. The study was conducted in a *P. falciparum* malaria holoendemic region, in Siaya County, western Kenya. Antibiotic susceptibility patterns of

the bacterial isolates were determined using disk diffusion according to the Clinical Laboratory Standards Institute guidelines where antibiotic resistance was defined as resistance of a microorganism to an agent to which it was previously sensitive. The results revealed that *Escherichia coli* had high resistance (80%-100%) to ampicillin/ sulbactum, gentamicin, and chloramphenicol, while non-typhodial salmonella (NTS) showed high resistance (71%-100%) to ampicillin/salbactum, amoxicillin/clavulanic acid, trimethoprim/sulfamethoxazole, chloramphenicol, nalidixic acid and ceftriaxone. Staphylococcus aureas displayed the least resistance (<41%) to ampicillin/sulbactam, gentamicin, amoxicillin/clavulanic acid, trimethoprim/sulfamethoxazole, and chloramphenicol. Further analysis showed that NTS resistance to chloramphenical was significantly higher in the acute visit group compared to malaria [+] and malaria [-] groups, (p=0.028). Additionally, NTS demonstrated higher resistance to ampicillin/ sulbactam in the acute illness relative to malaria [+] and malaria [-] groups (P=0.010). The current study shows that most bloodstream NTS, *E. coli* and *S. aureus* isolates are resistant to commonly used antibiotics and that NTS characterize acute illness in western Kenya.

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RESISTANCE OF *NEISSERIA GONORRHOEAE* IN REMOTE PERUVIAN JUNGLE SETTINGS

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Gonorrhea is a common sexually transmitted disease that if not treated may lead to chronic reproductive health complications, especially in women. Increasing rates of antibiotic-resistant *Neisseria gonorrhoeae* (NG) has become a public health concern worldwide and continued surveillance for NG is critical, especially in developing countries and underreported areas. We established a NG surveillance network in collaboration with two hospitals and a reference laboratory in the city of Iquitos located in the Peruvian Amazon. NG cultures of urethral and vaginal swabs were plated immediately on GC and Modified Thayer-Martin agar prior to transport to our nearby laboratory. Presumptive identification of colonies was determined by colony morphology, oxidase test, Gram stain, and biochemical identification with the API NH (Biomerieux) kit. Antibiotic susceptibility was determined by E-test (Biomerieux). From February 2013 to March 2016, a total of 189 cases were screened with 70/189 (37%) positive for NG. From 69 isolates with available susceptibilities, 49/69 (71%) and 32/69 (46%) of isolates showed resistance to penicillin and ciprofloxacin, respectively, whereas only 5/69 (7%) were susceptible to both antibiotics. Additionally, 25/69 (36%) showed resistance to tetracycline whereas 15/69 (22%) and 32/69(46%) exhibited intermediate susceptibility to penicillin and ciprofloxacin, respectively. No resistance was found for ceftriaxone, cefixime and azithromycin. Our results shows very high rates of fluoroquinolone resistance among NG in Iquitos, which is still widely used as first line therapy in Peru. The results highlight the need to update Peruvian MoH treatment guidelines to current U.S. Centers for Disease Control and Prevention (CDC) standards.

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PRINCIPLES, PRACTICES, AND KNOWLEDGE OF PROVIDERS EVALUATING CHILDREN PRESENTING WITH FEVER IN KENYA

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Clinicians in low resource settings where malaria is prevalent face many challenges in diagnosing and treating febrile illnesses in children. Given the change in WHO guidelines in 2010 recommending malaria testing prior to treatment in any child presenting with fever, clinicians are now required to expand the differential when malaria testing is negative. Prior studies have indicated that resource availability, need for additional training in differentiating non-malarial illnesses, and lack of understanding within the community of when to seek care play a role in effective diagnosis and treatment. We interviewed 20 clinicians (2 pediatricians, 1 medical officer, 2 nurses, and 15 clinical officers) working at 5 different government-sponsored public clinic sites (2 rural clinics, 1 urban clinic, and 2 larger referral hospitals) in two areas of Kenya where malaria is prevalent. Clinicians were interviewed one-on-one using a structured interview technique. Interviews were then analyzed qualitatively for themes. Themes included the following: 1) Primary reliance of history and physical exam in diagnosis of febrile illness; 2) Strong familiarity with IMCI guidelines and recognition of the "danger signs" but lack of comfort with the diagnosis and treatment of illnesses that deviate from the protocol; 3) Use of antibiotics as a fallback when diagnosis is unknown; 4) Difficulty with community understanding of febrile illness; 5) Lack of resources including diagnostics, medications, and training modalities. These themes persisted across the 5 sites, despite variation in levels of medical care. Within these themes, clinicians consistently expressed a need for reliable basic testing, especially hemograms and bacterial cultures. Providers discussed the use of counseling and education to improve community understanding of febrile illness in order to decrease preventable deaths in children. Our results suggest that since malarial testing has become more widespread, revisions to provider training and diagnostic tools are necessary to improve diagnosis and effective treatment of febrile illness in children in malaria endemic regions.

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HIGH PREVALENCE OF PARASITIC INFECTIONS AMONG RECENT IMMIGRANTS IN CHICAGO

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Extensive testing for infectious diseases is performed on refugees. This testing is not provided to the 42.1 million immigrants living in the US, many of whom share similar risk factors and migrated from countries where parasites are highly prevalent. We recruited 119 asymptomatic recent immigrants into a cross-sectional study to assess the prevalence of parasitic infections in this population. All 119 participants were asked about symptoms and provided stool and blood samples for Ova & Parasite exam, eosinophil count, and Immunoglobulin E (IgE) level; a subset of the samples (73/119) were randomly selected for additional parasite serologic testing. Enrolled subjects had a mean age of 33 years (SD 17.7) and 65 of the 119 subjects were female (55%). Subjects had immigrated primarily from Mexico (30/119, 25%), India (26/119, 22%), other Asian countries (30/119, 25%), and Central and South America (23/119, 19.3%). Twenty of 119 subjects (17%) tested positive for a parasitic infection and

4/20 subjects (20%) with parasites had multiple infections. The most commonly identified infections were: *Blastocystis hominis* (9/119 subjects, 7.6%), followed by *Toxocara* (6/73, 8.2%), schistosomiasis (5/73, 6.8%), strongyloidiasis (3/73, 4.1%), and Chagas disease (1/73, 1.4%). There was no difference in gender (p=.63) or recency of immigration (p=.23) between those with and without parasitic infections. Immigrants from Asian countries had a significantly higher likelihood of infection compared to the other regions (p<.001). IgE was significantly higher in those with a parasitic infection (GM 136.7 IU/mL) compared to those without (GM 65.53 IU/mL, p=.03), but mean AEC did not differ between the groups (p=.69). There was no difference in clinical symptoms between groups (all p values >.05). Based on our preliminary data, parasitic infections likely represent a large and as-yet-unidentified burden of disease in the immigrant population. The lack of symptoms or signs predictive of parasitic infections suggests immigrants might benefit from the same screening provided to refugees, as all of these treatable infections can have significant health impacts.

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THE MHC CLASS I CHAIN-RELATED MOLECULE A (MICA) 129 METHIONINE/VALINE DIMORPHISM ASSOCIATED WITH CHAGASIC MEGACOLON IN BOLIVIA

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Chagas disease, caused by the flagellate parasite *Trypanosoma cruzi* affects 5-6 million people mainly in Latin America and causes over 10,000 deaths per year according to the WHO. The underlying mechanisms that lead to the development of complications from chronic Chagas disease are not fully understood. To identify host genetic factors, we focused on the MHC class I chain-related molecule A (MICA) gene polymorphism, which could change the responsiveness of Natural Killer (NK) cells through its ligand. A single nucleotide polymorphism at residue 129 of the MICA gene change a single amino acid from strong binder (methionine) to a weak binder (valine) of the NKG2D receptor, a C-type lectin receptor expressed on effector cells including NK, αβ- and γδ-T cells. Recently MICA 129 met homozygote was associated to left ventricular systolic dysfunction (LVSD) in patients with Chagas Chronic heart disease. Therefore, we asked whether MICA 129 met/val polymorphism affects the clinical forms of Chagas disease in Bolivia. A total of 303 chronic Chagas patients, 80 cardiac, 99 megacolon, and 72 indeterminate forms, and 87 seronegative controls in Santa Cruz, Bolivia were diagnosed by electrocardiogram and Barium enema colon X-ray. MICA129 polymorphism (A > G, rs1051792) was genotyped by TaqMan Allelic discrimination assay. Here, we report that the MICA 129 A allele (methionine) was significantly decreased in frequency in the megacolon patients compare to indeterminate (OR=0.24, Pcorrected=0.0021) suggesting this allele is resistant against megacolon. Thus, the strong binding between NKG2D and MICA 129 met may be related to the protection against the tissue damage in the colon.

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INCIDENCE AND SPECTRUM OF HEALTH PROBLEMS AMONG TRAVELERS TO MYANMAR

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Nowadays, Myanmar becomes an attractive destination. The number of traveler visiting Myanmar is rapidly increasing every year. However, little is known about their pre-travel preparation and incidence of health problems during their trip. We, therefore, conducted a cross-sectional study at the arrival halls of Bangkok International airports. Travelers who just completed their trip in Myanmar were invited to fill the questionnaire. They were asked about their demographic profile, pre-travel health preparations, and their health problem during their stay in Myanmar. From March to December 2015, 397 questionnaires from Thais and 301from foreigners were collected and analyzed. 48.7% of travelers were male, and the median age was 37 years in both groups. Among foreigner group, most of them were from Europe (73%). Up to 82% of foreigners sought pre-travel health information before their trip, while only 36.5% of Thais did so. The main reason for travel was tourism in 91% of foreigners while only 58.1% of Thais traveled for tourism. Foreigners were more likely to travel as backpackers, and engaging in outdoor activities such as trekking, cycling or swimming than Thais. There was also significant difference in the average length of stay between foreigners and Thais (21.7 days vs 7.08 days, p<0.001). Overall health problems were reported in 29.2% of foreigners, the most common being diarrhea which reported in 22.3% of foreigners followed by upper respiratory tract symptoms, fever, and skin problems. While only 12.6% of Thais reported some health problems. The most common one was upper respiratory tract symptoms followed by diarrhea, fever and skin problem. Most health problems were mild and self limited in both groups. However, six foreign travelers had to visited doctor and two had to be admitted. While only one Thai traveler had to visit a doctor in an out-patient department. In conclusion, health problem was fairly common among travelers to Myanmar. Nearly 30% of foreign travelers reported some health problems. Most health problems were mild and spontaneous recovery; however up to 2% of foreign travelers need to visit a doctor while traveling in Myanmar.

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EVALUATION OF A BOOSTER DOSE OF ROTAVIRUS VACCINE GIVEN CONCOMITANTLY WITH MEASLES AND YELLOW FEVER VACCINES IN MALIAN INFANTS

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Rotavirus vaccines, administered early in the 1st year of life, are modestly effective in low resource countries and immunity may wane. Rotavirus is still a major cause of moderate to severe diarrhea in the 2nd year of life, and a booster dose of rotavirus vaccine might extend protection. We evaluated whether a booster dose of pentavalent rotavirus vaccine given to 9-11 month old Malian infants would interfere with immune responses to routine vaccines. Infants were randomized 1:1 to receive PRV or no PRV co-administered with measles and yellow fever vaccines. Serum was

collected before and 28 days after vaccination. Anti-measles IgG was measured by ELISA; seroconversion was defined as a positive result among baseline seronegatives. Yellow fever neutralizing antibodies were measured by plaque reduction; seroresponse was defined as >4-fold increase in post-vaccination titer. Noninferiority (<10% difference in seroconversion or seroresponse rates) for measles and yellow fever were co-primary objectives. Anti-rotavirus IgA and IgG concentrations were measured by ELISA. From October 15, 2014 to December 18, 2014, 600 infants were enrolled with 300 receiving PRV. 513 were baseline measles seronegative with 255/261 (97.7%) (PRV group) seroconverting compared to 246/252 (97.6%) (no PRV group); difference, 0.1% (95% CI, -4.0 to 4.2). In the yellow fever analysis, 141/293 (48.1%) (PRV group) seroresponded compared to 153/293 (52.2%) (no PRV group); difference -4.1% (95% CI, -12.2 to 4.0). However, with yellow fever seroresponses defined as >2-fold rise, 202/293 (68.9%) (PRV group) seroresponded compared to 206/293 (70.3%) (no PRV group); difference -1.4% (95% CI, -8.8 to 6.1). Post-vaccination anti-rotavirus IgA and IgG geometric mean concentrations rose to 118 (95% CI, 91 to 154) and 364 (95% CI, 294 to 450) in the PRV group compared to 68 (95% CI, 50 to 92) and 153 (95% CI, 114 to 207) in the no PRV group. Concomitant administration of PRV did not appear to interfere meaningfully with immune responses to MV and YFV and substantially increased rotavirus antibody levels. Clinical benefit of a booster dose of PRV in the 2nd year of life should be studied.

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AN OPTIMIZED AGE BASED DOSING REGIMEN FOR SINGLE LOW DOSE PRIMAQUINE FOR TRANSMISSION BLOCKING IN CAMBODIA

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In 2012, the WHO recommended the addition of single low single low (0.25 mg base/kg body weight) dose primaquine (SLDPQ) to artemisinin based combinations to block the transmission of *Plasmodium falciparum* without testing for glucose 6 phosphate dehydrogenase deficiency. The targeted group was non pregnant patients aged ≥ 1 year (later changed to ≥ 6 months) with acute uncomplicated falciparum malaria, primarily in countries with artemisinin resistant *P. falciparum* (ARPf). No dosing regimen was suggested. We, therefore, designed a user friendly, age based, SLDPQ regimen for Cambodia, the country most affected by ARPf. By reviewing PQ's pharmacology, we defined a therapeutic dose range of 0.15-0.38 mg base/kg (9-22.5 mg in 60 kg adult, therapeutic index 2.5). Primaquine doses (1-25 mg) were tested using a modelled, anthropometric database of 28,138 Cambodians (23,338 healthy individuals, 4,199 with malaria, and 1,292 other infections); age distributions were: < 5y [19.35% (n=5,383)], 5 to 17y [20.37% (n=5,469)] and adults [59.65% (n=15,531)]. Optimal age dosing groups were selected according to calculated mg base/kg doses and proportions of individuals receiving a therapeutic dose. Four age dosing bands were defined: (i) 6m-4y, (ii) 5-9y, (iv) 10-14y, (v) ≥ 15y to receive 2.5, 5, 7.5 and 15 mg of PQ base, resulting in therapeutic doses in 97.41% (5,494/5,640), 90.53% (1,511/1,669), 97.68% (1,473/1,508), and 95.69% (18,489/19,321), respectively. Corresponding median (1st, 99th centiles) mg base/kg doses of PQ base are: (i) 0.23 (0.15-0.38), (ii) 0.29 (0.18-0.45), (iii) 0.27 (0.15-0.39), and (iv) 0.29 (0.2-0.42). In conclusion, this SLDPQ regimen could contribute substantially to malaria elimination and requires urgent evaluation in Cambodia and other Greater Mekong Subregion countries with similar anthropometric characteristics. It also guides primaquine manufacturers on suitable tablet strengths and doses for paediatric friendly formulations. Development of similar age based dosing recommendations for Africa is needed.

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COMMUNITY HEALTH WORKER'S SOCIO-DEMOGRAPHIC CHARACTERISTICS AND ICCM PERFORMANCE IN RWANDA

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The health of children under five remains worrying in developing countries. Indeed, nearly ten million children still die annually before reaching the age of five. Since 2008, community health workers (CHWs) in Rwanda have been trained to carry out integrated community case management (iCCM) in children under five. We carried out this study in 2014 to determine the effect of socio-demographic characteristics on CHWs performance. A cross-sectional study was conducted in 25 districts in Rwanda. Data was collected using a structured questionnaire and client satisfaction. We used STATA for data analysis. Descriptive statistics were calculated. Odds ratios (OR) and 95% confidence intervals (CI) for the predictors of the performance were calculated. Relationships were determined using logistic regression. Results A total of 19,402 CHWs were observed delivering iCCM package among sick children. Of these CHWs, 51.2% (10,075) were females. The level of performance was estimated to 87.6% for malaria treatment, 88.7% for pneumonia treatment, 85.4% for diarrhea treatment, 76% for malnutrition assessment, 28.1% for counseling on diseases prevention, and 99% for detection of danger signs. The study showed significant relationships of sex with pneumonia treatment. Females CHWs were 1.08 more likely to treat pneumonia than males (OR=1.089, 95%CI: 1.027-1.156). In addition, CHWs aged 35 and above are 1.09 more likely to give more advices on disease prevention than other age group (OR=1.098, 95%CI: 1.029-1.173). CHWs with at least one year of secondary school were 1.06 and 1.08 more likely to treat malaria correctly (OR=1.062, 95%CI: 1.024-1.101) and pneumonia (OR=1.088, 95%CI: 1.050-1.124) when compared to CHWs with education level of primary school. Conclusion Socio-demographic characteristics of CHWs in Rwanda affect iCCM performance in different ways. We recommend targeted interventions and appropriate intensive refresher training for CHWs on iCCM package.

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PATIENT PERCEPTIONS OF TRACHOMATOUS TRICHIASIS SURGERY IN THE FAR NORTH REGION OF CAMEROON

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In Cameroon, trachoma mapping conducted in 2010-2011 identified 13 health districts (HDs) in the Far North region with a prevalence of trachomatous inflammation-follicular (TF) of over 10% in children aged 1-9 years. Out of these HDs, 8 were identified with trachomatous trichiasis (TT) over 1% in persons aged 15 and over in the population. Surgery is an important component of the SAFE (Surgery, Antibiotic treatment, Facial cleanliness and Environmental improvement) strategy to achieve trachoma elimination. Cameroon conducted the TT surgery campaign in the region with the support of the United States Agency for International Development's MMDP Project, managed by Helen Keller International. Six

months following the TT surgery campaign during which 1,080 patients were operated, a patient follow-up survey was conducted to evaluate patients' perception of the surgery and surgery outcomes. A convenience sampling of 213 patients that received TT surgery in the previous 6 months was realized and a questionnaire was administered to assess patients' perception and satisfaction. Median age of patients at surgery was 60 years. 126 (59.2%) of the surveyed patients were female and 86 (40.4%) were male. The following outcomes were recorded: 10 (4.7%) returned to the hospital because of the sand sensation in the operated eye, 6 (2.3%) returned due to excessive tearing and 16 (7.5%) returned for eye pain. The following patients' perceptions were recorded: 210 (98.6%) reported improved vision, 205 (96.2%) patients with less difficulty in performing daily activities after surgery, 210 (98.6%) patients would recommend surgery to others, 211 (99.1%) patients with less pain after surgery. Patient perception on TT surgery is one way to evaluate the quality of surgery in order to help investigate on surgery refusals and improve the quality of care. The results showed a successful TT surgery campaign in the region.

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MEASURING THE EFFECT OF SOIL-TRANSMITTED HELMINTH (STH) INFECTIONS ON COGNITIVE FUNCTION IN CHILDREN: SYSTEMATIC REVIEW AND CRITICAL APPRAISAL OF EVIDENCE

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Recently the role of STH infections in cognitive developmental impairment of children has been under intense scrutiny. We conducted a systematic review of the evidence for associations between STH infections and cognitive function of children. We aimed to summarise the effect size by domains of cognitive function in three age strata (i.e. children <24 months; children 24-59 months and school-aged children). Using the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) protocol we searched relevant databases including PubMed, CINAHL, EMBASE, Medline via Web of Science, Cochrane, ScienceDirect, Psych Articles and PsychINFO via APA PsychNET and Scopus. A total of 42 papers fulfilled the inclusion criteria for the systematic review; these include 10 studies from a recent Cochrane review. We next conducted a critical appraisal of the variability of cognitive function measurement tools used to assess the effect of STH infections on different domains of cognitive function of children of different age groups. Our findings demonstrate remarkable variation in tested domains and lack of consistency in the use and analysis of measurement tools. Cognitive function measures in children under five years of age has been mainly limited to domains of gross motor, fine motor and language skills, whereas in school-aged children most studies tested domains such as memory and processing speed. Even within the same age group the results on the association between STH infections and measures of cognitive development were often conflicting. This study demonstrates the need for the establishment of methodological consensus in the deployment and analysis of adequate measurement tools to detect the effect of STH infections on different domains of cognitive function in children at different ages. This will be an imperative next step to improve study validity and generate conclusive evidence of the role of STH infections in cognitive development in children.

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VARICELLA ON SHIPS: IS THERE A RISK TO TRAVELERS?

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Masters of ships with U.S. ports of call are required to report certain illnesses to CDC Quarantine Stations. We describe varicella case reports recorded in CDC Quarantine Activity Reporting System from January 2010 to February 2016. Denominators vary according to the number of cases for which data were available. There were 1,000 reports of varicella from ships during the study period. Most case-patients were male (79.79%, 525/658) and older than age 15 years (89.66%, 503/561). Cruise ships reported 93.30 (933/1,000) of cases, and 6.30% (63/1,000) of cases were reported by cargo ships. On cruise ships and cargo ships, 83.82% (782/933) and 98.41% (62/63) of cases, respectively, were in crew members. Crew member cases were most often from the Philippines (23.46%, 99/422), Indonesia (18.72%, 79/422), and India (17.06%, 72/422). Passenger cases were most often from the United States (21.95%, 18/82), the United Kingdom (10.98%, 9/82), Canada (8.54%, 7/82), and Sweden (8.54%, 7/82). Case-patients typically presented with a rash (99.80%, 998/1,000) and fever (54.30%, 543/1,000). Cases were reported every month of the year. Cruise ship case-patients were isolated, on average, 0.65 days (n = 292, SD 0.96) after rash onset and reported to CDC, on average, 2.32 days (n = 793, SD 4.66) after rash onset. Cargo ship case-patients were isolated, on average, 1.15 days (n=20, SD 1.39) after rash onset and reported to CDC, on average, 5.17 days (n=59, SD 9.56) after rash onset. Varicella cases resulted in hospitalization in 3.52% (20/568) of reports; one case was fatal (0.10%, 1/1,000). Crew members on cargo and cruise ships frequently are from tropical regions where there usually is a higher susceptibility to varicella among adults, or varicella vaccination is not routinely provided. A majority of cases reported were crew members, who, as adults, are at higher risk of complications due to varicella. Given that exposure to varicella on ships can occur year round, varicella vaccination prior to boarding a ship is advisable for those without evidence of immunity.

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SPLENOMEGALY IN CONGOLESE REFUGEES APPLYING FOR RESETTLEMENT FROM UGANDA TO THE UNITED STATES

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In 2014, the International Organization for Migration (IOM) reported a high number of United States (US)-bound Congolese refugees from Uganda with splenomegaly of unknown etiology. In March and July 2015, refugees with splenomegaly on physical examination were offered evaluation and treatment, including abdominal ultrasonography and laboratory testing. Among 987 persons screened, 145 (14.7%) had splenomegaly and received further testing. Of the 144 who had abdominal ultrasound, 122 (84.7%) had massive splenomegaly (defined as >4 a standard mean for splenic size adjusted for height), 135 (93%) had normal liver architecture, and 8 (5.5%) had hepatic nodules/masses.

Thirty-nine (26.9%) were positive for malaria by RDT® (Bioline HRP2/Pan LDH); all tested negative by thin blood smear (thick films were not performed). Eighty-six percent (135/145) were positive for PFMSP-1 IgG (indicating past exposure to *Plasmodium falciparum*). Three (2.1%) tested positive for *Schistosoma mansoni* ova by stool wet prep. Urine ova and parasite were negative. All tested negative for *Leishmania* by rK39 testing, but 1 (0.7%) was positive by serology. Five (3.5%) had positive HBsAg, and 10 (6.9%) had detectable HCV antibodies. Refugees with palpable splenomegaly were treated with antimalarials at the time of diagnosis, and all refugees are given presumptive treatment with artemether-lumefantrine prior to departure to the US - thus those with splenomegaly received two treatment courses. CDC issued recommendations to US providers to conduct additional laboratory and radiology testing. Due to concerns of malaria as an etiology, and reports of non-falciparum malaria in this population, treatment for hypnozoites with primaquine (after glucose-6-phosphate dehydrogenase testing) following arrival in the United States was recommended. Pre- and post-malaria treatment total IgM is currently pending. This initial evaluation did not identify a definitive unifying etiology of splenomegaly and while pending results may indicate a likely etiology, further study of this prevalent condition in Congolese refugees in western Uganda is needed.

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IMPROVING THE QUALITY OF CARE FOR COMMON CHILDHOOD ILLNESSES AMONG PATENT AND PROPRIETARY MEDICINE VENDORS IN EBONYI STATE, NIGERIA

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In Nigeria, malaria, pneumonia and diarrhea are the leading causes of death in children under five. As patent and proprietary medicine vendor (PPMV) shops are the origin of a high proportion of treatments, they could be a strategic partner in increasing the proportion of children that receive appropriate treatment for common childhood illnesses. MalariaCare and the Expanded Social Marketing Project in Nigeria, in collaboration with the Federal Ministry of Health (FMOH) and Ebonyi State Ministry of Health, are conducting a pilot evaluation to assess the ability of trained PPMVs to manage cases of common childhood illnesses according to national health standards. The pilot intervention includes integrated community case management training, covering the use of RDTs, ACTs, ORS, zinc, respiratory timers and amoxicillin, followed by on-site mentoring; and targets poor-performing, high-volume PPMV shops for more cost-effective resource allocation. The evaluation is a quasi-experimental study with 2 intervention and 2 control areas in Ebonyi State. At baseline, we conducted household and outlet surveys to determine the quality of care provided by PPMV shops. Household surveys identified 2,614 children with fever, diarrhea or pneumonia symptoms; 83% had fever, 24% percent had diarrhea, and 4% reported pneumonia symptoms. In 37% of cases treatment was received from a PPMV shop. Of these, 13% of fever cases received an ACT, 1% of diarrhea cases received ORS and zinc, and 2% of pneumonia cases received amoxicillin. Among cases treated at a PPMV shop (68%), rather than treating at home with products previously purchased from PPMVs (32%), only 5% of fever cases had a diagnostic test and 9% of pneumonia cases had their respiratory rate checked. Of the 198 PPMV providers included in the outlet survey, 65%, 4%, and 21% knew the correct first line treatment for malaria, diarrhea and pneumonia, respectively; 67% were not aware of RDTs. End line evaluation results that demonstrate whether the pilot was effective in improving the proportion of children under five with common childhood illnesses receiving appropriate treatment will be presented.

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HIGH PROTEIN DIETARY SUPPLEMENT IMPROVES NUTRITIONAL STATUS AND RECOVERY IN CHILDREN WITH BURKITT'S LYMPHOMA: A NON-RANDOMIZED CONTROLLED STUDY

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Each step of the cancer continuum from diagnosis to recovery poses nutritional challenges. In children with cancers, poor nutritional status will lead to faster disease progression, slower recovery and poorer survival. The effect of nutritional intervention using a soya milk powder (SMP) supplement on nutritional status, recovery and mortality among children undergoing chemotherapy for Burkitt's lymphoma (BL) was studied. Sixty-four subjects were recruited for this non-randomised controlled intervention study. The intervention group was provided the supplement measured to provide 80% RDA for protein and was followed for 6 months, taking measurements at the 0, 3 and 6-months follow-up. Baseline characteristics of the study population were similar except for haemoglobin and prealbumin. SMP supplementation was associated with reduced prevalence of malnutrition (<-2 sd BMI-for-age) from 50% baseline up to 0% six months after the intervention (p=0.005). Likewise, the SMP improved anaemia status (100%, 76.9% and 15.8% anaemia between baseline, 3-months and 6-months follow up respectively), serum zinc deficiency (87.5%, 50% and 52.6%, p=0.004) and reduced glutathione deficiency (GSH) (21.9%, 0 and 0, p=0.045). Same cannot be said in the non-intervention group. Recovery from BL 1 year after the intervention was 47% compared with 16% in the non-intervention, whereas mortality was 19% versus 28% between the intervention and non-intervention groups respectively. Dietary supplementation using a high protein-based SMP improved nutritional status, recovery and survival in children with BL hence the need for larger studies to assess the efficacy of nutritional intervention as non-conventional treatment of childhood cancers in limited resource settings.

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DENGUE AND LEPTOSPIROSIS CO-INFECTION IN THE PERUVIAN AMAZON, 2013-2014

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Dengue is a major public health problem associated with high morbidity and mortality rates in developing countries. In Iquitos, the largest city in the Peruvian Amazon, dengue and leptospirosis are responsible for 40% and 30% of acute undifferentiated febrile illness (AUFI) cases, respectively. Despite this data, few studies have discussed about co-infection, which could explain some atypical or severe cases. Passive clinic-based surveillance conducted by NAMRU-6 in 12 Iquitos health facilities, provided an opportunity to evaluate the clinical impact of leptospirosis infection in dengue confirmed cases (co-infections). From January 2013 to December 2014, 4,211 AUFI patients enrolled in our study provided acute and convalescent blood samples, and were evaluated clinically and epidemiologically. A dengue case was defined as any patient where dengue virus or viral RNA was detected through real -time PCR or viral isolation in an acute sample, or demonstrated a seroconversion (4-fold increase IgM antibodies) between acute and convalescent samples. Leptospira infection was defined by seroconversion of IgM antibodies plus the presence of a titer of Microscopic Agglutination Test (MAT) in convalescent sample of ≥1/400 or a titer of MAT≥1/800 in any of the

paired sample. Of the febrile cases where complete dengue and leptospira testing and clinical data was available, 1,533 of 1,572 (97.5%) had a confirmed dengue infection. Of these, 39 (2.4%) cases had leptospira co-infection. None of the clinical findings (constitutional, gastrointestinal and respiratory symptoms) or complications were associated to co-infection ($p > 0.05$, Pearson Chi square test). The hospitalization rate and shock syndrome ($p>0.05$) were similar in both groups with no fatal cases. Asymptomatic infections due to leptospirosis occur commonly in this city and may explain this finding, even though both dengue and leptospirosis have been associated with severe and fatal disease in Iquitos. In dengue and leptospirosis endemic areas, serological results should be interpreted in the context of the clinical presentation.

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A PROSPECTIVE ASSESSMENT OF ANTIBIOTIC PRE-TREATMENT, USING A URINE ANTIBIOTIC BIOASSAY, AMONG PATIENTS ATTENDING AN INFECTIOUS DISEASE HOSPITAL IN MANILA AND THE RELATIONSHIP BETWEEN ANTIBIOTIC USE AND SOCIOECONOMIC STATUS

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The widespread unregulated use of antibiotics contributes to the rising prevalence of antibiotic resistance in Southeast-Asian countries. Antibiotic usage before a medical consultation also reduces the possibility of identifying the casual pathogen due to the reduced sensitivity of bacterial culture. We conducted a hospital-based observational study investigating prior antibiotic usage in patients attending San Lazaro Hospital (SLH) for a medical consulation. SLH is the National Infectious Disease Hospital and provides free-health care to a low-income population in Metro Manila, Philippines. Patients attening the ER with a history of fever were enrolled. A urine bioassay was used to detect antibiotic activity in urine using three organisms: *Bacillus stearothermophilus* (ATCC7953); *Escherichia coli* (ATCC25922); and *Streptococcus pyogenes* (ATCC19615). Patients or caregivers reported their medication history, clinical information and socioeconomic status. During the study period (2nd February 2015 to 2nd July 2015) 410 patients were enrolled and provided a urine. The median (IQR) age was 14 (7 to 23) years and 158 (39%) reported prior antibiotic use, predominantly a beta-lactam antibiotic. A total of 164 (40%; 95%CI 35 to 45) patients were positive by urine bioassay with any of three organisms. The Bacillus assay detected 162 (99%; 95%CI 96 to 100) cases. Many patients with a positive urine bioassay were clinically considered to have dengue (n=91, 55%; 95%CI 48 to 63). Patients with a positive bioassay were significantly more likely to be from the lowest-income group (AOR 1.7; 95%CI 1.1 to 2.6) and require hospital admission (AOR 2.1; 95%CI 1.3 to 3.5). Antibiotics are widely used in the community setting in Manila by fever patients before the medical consultation, and this antibiotic use is significantly more common among those with the lowest-income.

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DIGITAL GANGRENE DUE TO RICKETTSIAL VASCULITIS - UNCOMMON MANIFESTATION OF AN UNDERAPPRECIATED DISEASE

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Rickettsiae are a common but underdiagnosed cause of acute febrile illness in India. Indian tick typhus caused by *Rickettsia conorii indica* belongs to the spotted fever group of *Rickettsiae* and is less frequently reported than scrub typhus (caused by *Orientia tsutsugamushi*). A healthy 45-year-old woman presented with fevers, chills, dysuria, vomiting, and headache. She was admitted to a community hospital and started on broad spectrum antibiotics. After failing to improve, she was transferred to our institution. On presentation she was febrile, hypotensive, tachycardic and tachypneic. She appeared critically ill with physical examination significant for periorbital edema, conjunctival suffusion and bibasilar rales. She was admitted to the ICU. Laboratory values showed anemia, thrombocytopenia, abnormal LFTs, proteinuria and lactic acidosis. Initial workup was negative for bacteremia, malaria, dengue, leptospira, typhoid fever and HIV. Weil-Felix titers were inconclusive. A tentative diagnosis of rickettsial infection was made and doxycycline was added. After 48 hours the patient improved and was transferred to the medical floor, but began to develop gangrene of the toes and fingers. Repeat testing showed persistent thrombocytopenia, abnormal LFTs and prolonged APTT. Repeat Weil-Felix OX-2 titer was 1:160, supporting a diagnosis of spotted fever group rickettsial infection. The patient was discharged home but there was no improvement in the condition of her digits on follow-up. *Rickettsiae* invade endothelial and smooth muscle cells of the microcirculation causing increased vascular permeability and microthrombi with resulting multi-organ dysfunction and thrombosis. While multi-organ dysfunction is common, gangrene is a rare complication. The epidemiology of Indian tick typhus is poorly defined, but serological surveys and case reports suggest widespread occurrence across the Indian subcontinent. It is characterized by a purpuric rash and unlike scrub typhus, an eschar is rarely seen. Early diagnosis and treatment with doxycycline is crucial to reduce morbidity and mortality due to *Rickettsiae*.

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ADVERSE EVENTS FOLLOWING PURIFIED CHICK EMBRYO CELL (PCEC, RABAVERT®) VACCINE IN THE VACCINE ADVERSE EVENT REPORTING SYSTEM, 2006 - 2015

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In 1997, the Food and Drug Administration licensed Purified Chick Embryo Cell (PCEC, RabAvert®) vaccine against human rabies. A previous post-licensure study in the Vaccine Adverse Event Reporting System (VAERS) during 1997-2005 did not find any safety concerns. This study was undertaken to assess the safety of PCEC vaccines in VAERS during 1/1/2006–12/31/2015. We searched the VAERS database for US reports of adverse events (AEs) among persons who received PCEC during 1/1/2006–12/31/2015. We reviewed all serious (those resulting in death, life-threatening illness, hospitalization, prolongation of existing hospitalization, or permanent disability) and accompanying medical records. Physicians assigned a primary clinical category to each reviewed report. During the study period, VAERS received 490 reports following PCEC vaccination; 38 (7.8%) were serious. No deaths were reported. Females accounted for 301 (61.4%) reports. PCEC was given alone in 407 (83.1%) reports. The median age was 28 years (range 0 - 88 years). The median time from vaccination to onset of an AE was ,<1 day. The most frequently reported AEs were headache (99; 20.2%), nausea (19.0%), pyrexia (91; 18.6%), dizziness (65; 13.3%), and pain (59; 12.0%). Among serious reports, the most common diagnostic categories were general disorders and administration site conditions (18;47.3%), and nervous system disorders (10; (26.3%). In conclusion, a review of VAERS reports did not identify any new or unexpected safety concerns for PCEC vaccines.

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TRICHOSTRONGYLUS INFECTION IN ITALY: REPORT OF FOUR AUTOCHTHONOUS OUTBREAKS DIAGNOSED IN A SINGLE CENTER

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Trichostrongylus spp are nematodes of herbivores, with a worldwide distribution. Humans get occasionally infected through the ingestion of vegetables and water contaminated with the larvae. Hence, the infection is more frequent in people in close contact with livestock and/or with vegetable gardens fertilized with organic manure. In industrialized countries, human infection has been rarely reported. At the Center for Tropical Diseases of Negrar (Verona), Italy, four outbreaks of trichostrongyliasis were diagnosed between August 2010 and July 2015. The four clusters occurred in three different Italian regions: Lombardy (Varese province and Brescia province), Piedmont, and Veneto. All outbreaks affected several/all members of the same household. Of the 12 patients involved, four had no symptoms. The others reported symptoms of different intensity, mostly abdominal pain (8 of 12 patients, 67%) and diarrhea (5 patients, 42%). Some also complained of myalgias (three), pruritus (two), nausea (one), and fever (one). Eosinophilia was present in all subjects, with or without symptoms, and was severe (>5000 eosinophils/mmc) in 5 patients. Medical history revealed in all cases the ingestion of vegetables exposed to sheep/goat manure. *Trichostrongylus* eggs were found at stool examination of only 4 patients. All patients from Piedmont were negative at stool examination, but the goat manure resulted positive for *Trichostrongylus* larvae. The patients were treated either with pyrantel pamoate (750 mg tablets, 4 tablets stat dose) or albendazole 400 mg x 2 for 10 days. Three patients with no symptoms preferred to defer treatment. All treated patients but one (complaining mild symptoms one year and a half after treatment) reported resolution of symptoms. A decrease in the eosinophil count was observed for all patients, including the ones who were not treated. Although not common in areas of temperate climate, *Trichostrongylus* infection should be considered in case of eosinophilia, in particular when present in different members of the same household.

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CLINICAL CHARACTERISTICS OF EBOLA SURVIVORS 40 YEARS POST INFECTION IN THE DEMOCRATIC REPUBLIC OF CONGO

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Ebola virus disease (EVD) is associated with a mortality rate ranging from 25 to 90% and to-date no cure or approved vaccine is available to the general public. The first cases of EVD were reported in 1976 in Yambuku, the north-eastern part of Democratic Republic of Congo (DRC-former Zaire) in the current province of Mongala. During this outbreak, 318 cases of EVD were recorded with 280 deaths, and 38 confirmed survivors. EVD is hypothesized to put suvirors at increased risk of adverse health courses, however, there is limited information regarding long-term health consequences .We enrolled 11 survivors 40 years after the Yambuku outbreak based on original line listings and case data from the DRC Ministry of Health. After informal consent was obtained, we collected information on clinical profiles at 3 times points: prior, during, and present day. The youngest survivor (15 at time of outbreak) is now 55 years old, and the oldest (46 during the outbreak) is 86 years old, with a mean age of 67.25 years. Four survivors were women and 7 men. The mean heart rate was 92 beats per minute with a peak of 128 beats per minute. The average systolic blood pressure was 142mmHg with a peak of 162mmHg. The most common symptoms reported were: muscle pain 82%, headache 64%, joint pain and fatigue 55%, continual cough 36% and ocular problems 27%. Our results add to the small body of the scientific literature that suggest EVD survivors may be at increased risk for long-term sequelae. Additional research with age and sex matched controls is needed to correlate current symptoms with EVD. Understanding the long-term consequences of EVD could lead to improvements in patient care and a better understanding of virus pathogenesis.

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EOSINOPHILIA IN PATIENTS PRESENTING TO A LARGE TRAVEL CLINIC: SPECTRUM OF DISEASE AND IMPLICATIONS FOR A SYSTEMATIC DIAGNOSTIC WORK-UP

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Eosinophilia is an immunological host response to a variety of triggers - ranging from allergens and malignancy to helminthic infections. Patients presenting to travel clinics with eosinophilia present a challenging task to physicians, as the underlying disease spectrum is as heterogeneous as the patient population and no standardized diagnostic work-up exists. In this study, we describe the spectrum of diseases in patients presenting to our travel clinic with eosinophilia. Additionally we assessed whether additional patients' characteristics or laboratory work-up could inform in order to define a more rational and effective diagnostic work-up. We analyzed data from 6,622 consecutive patients between September 2007 and May 2014. Eosinophilia, defined as an absolute eosinophil count of >500 /µl, was present in 2,4% (155/6,622). The median age was 33 years (range: 5-77 years) and 40% (60/155) were female. Most patients presented as ill-returning travelers (78%, 121/155), others were classified as immigrants, expatriates or tourists visiting Germany. An infectious cause was detectable in 57% (89/155) - with the most common specific infections being schistosomiasis (19%, 29/155) and strongyloidiasis (8%, 12/155). Atopic disease and asthma accounted for 10% (16/155), while no specific cause could be identified in 14% of patients (21/155). Patients with a confirmed infectious cause of eosinophilia had slightly higher eosinophil counts (835 vs 712 /µl, p=0.04) but equal levels of IgE (152 vs 176 kU/L, p=0.70) compared to those without an infectious cause. In travelers who visited friends and relatives and in immigrants, an infectious cause was identified more often than in classical tourist travelers (p<0,01). In conclusion, eosinophilia is an important condition in patients presenting to a travel clinic. Our data show that IgE did not yield any added diagnostic value. Instead a systematic diagnostic approach could help to identify the majority of underlying conditions.

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MALARIA AND LASSA FEVER VIRUS CO-INFECTION IN SOUTHERN MALI

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Lassa Virus (LASV) was unknown in Mali until an exported case of Lassa fever (LF) was reported in 2009. Since then, multiple rodent surveys have been conducted and shown evidence of LASV infected *Mastomys natalensis* in several communities restricted to the southern tip of the country near the border of Côte d'Ivoire. LF has similar clinical manifestation with malaria and maybe misdiagnosed. To date only a single case of LF has been confirmed in Mali and little is known about the

prevalence of LASV exposure in human population. The goal of this study is to determine the prevalence of LASV exposure and malaria parasite prevalence in three villages in southern Mali, where infected rodents have been documented and where malaria is endemic. About 1 ml of blood was drawn by technicians for haemoglobin measurement and *Plasmodium* detection by standard microscopy of blood smears from 600 participants under a light microscope for evidence of malaria infection and parasite quantification and an enzymelinked immunosorbent assay (ELISA) was used to screen serum samples for the presence and quantification of Lassa fever virus IgG and IgM. The overall IgG sero-prevalence of LFV was 33.2% and the IgM 1.27% (n =600). The IgG sero-prevalence was as high as malaria parasite prevalence in Bamba (44.0%, vs 40.0%) and soromba (41.0% vs 48.5), and lower in Banzana (14.5 vs 45.0). The majority of malaria infection was due to *P. faciparum* (95.1%). Our results show for the first time that LASV is circulation in human population of Southern Mali and the prevalence of LASV exposure is as high as malaria parasite prevalence. More investigation is needed to identify and monitor hot spot areas which are potential epidemic prone areas.

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PROGNOSIS PREDICTION FOR PATIENTS WITH CONFIRMED EBOLA VIRUS DISEASE IN SIERRA LEONE AND LIBERIA: CLINICAL IMPLICATIONS AND FUTURE APPLICATIONS

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We used a computational framework called FMA to derive prognosis prediction models for confirmed Ebola Virus Disease (EVD) patients, with demographic information, clinical symptoms, and Cycle Threshold (CR) values from RT-PCR as covariates. Our goals are (1) to determine which are the factors that better prognosticate patient survival upon presentation, and (2) to explore the possibility of improved patient triage in emergency settings based on the predicted risk scores. We obtained retrospective cohort data from a total of 476 confirmed EVD patients admitted to five Ebola Treatment Units (ETU) in Sierra Leone and Liberia, between 2014 and 2015. Data was recorded as part of routine clinical care at each ETU, and was combined and deposited in International Medical Corps' secure database. The overall mortality in this cohort is 58%, and univariate regression analysis yields the following variables with a P-value under 0.05: CT measurement in first day (P=8×10-9, OR=0.28), Age (P=9×10-3, OR=1.15), Jaundice (P=10-2, OR=3.9), and Hemorrhagic eyes (P=4.5×10-2, OR=0.65). We trained a Logistic Regression model with Elastic Net regularization on these variables, augmented with a number of additional clinical factors above P=0.05: Coma (P=6.2×10-2, OR=undefined as all patients with coma died), Confusion (P=7.1×10-2, OR=3.1), Breathlessness (P=7.7×10-2, OR=1.5), and Headache (P=10-1, OR=0.7). The CT, Coma, and Confusion variables have missing entries for 60% of the patients. We addressed this issue by using Multiple Imputation (MI) to complete the records, and observed that mortality of patients with missing values is similar to that of the entire dataset, which indicates that the data is Missing Completely At Random (MCAR). Our best model has non-zero coefficients for CT (OR=0.89), Age (OR=1.006), Jaundice (OR=2.7), Hemorrhagic eyes (OR=0.67), Coma (OR=3.7), and Confusion (OR=3.2). The AUC of this model is 0.74, with a 95% CI of (0.74, 0.86), and it shows low overfitting and good calibration. Decision curve analysis suggests that interventions based on this model would be better than a treat-all policy for decision thresholds above 0.45.

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COMMON FINDINGS AND EXCLUSIONS DURING ENROLLMENT OF ADULT MALIAN VOLUNTEERS FOR MALARIA VACCINE STUDIES

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For malaria vaccine testing, volunteers must be enrolled from a suitable site. Screening is a critical step to identify and enroll healthy volunteers. We conducted two malaria transmission blocking vaccine studies in Malian adults in Bancoumana and surroundings from 2013 to 2015. Once informed consented is obtained, each individual undergoes clinical and laboratory assessment. This study aimed to assess the frequency of different conditions or findings that preclude the enrolment of adult subjects into vaccine studies. In 2013, we screened 277 and enrolled 120 volunteers into the malaria vaccine study of Pfs25H-EPA/Alhydrogel®. The eligibility rate was 43.3 % (120/277), and 14.1% (39/277) of volunteers withdrew their consent before or at the time of enrollment. The most frequent reasons for screening failure were positive Hbs antigen (6.9%, 19/277) and neutropenia (6.1%, 17/277). HIV frequency was 3.6% (10/277). No differences were seen among villages for causes of screening failure. In 2015, we screened 478 volunteers and the eligibility rate was 47.1 % (225/478), with 21.3% (103/478) withdrawing consent before or at the time of enrollment. The most frequent reasons for screening failure were positive Hbs antigen (8.8%, 42/478), HIV (7.5%, 36/478) and positive HCV (4.0%, 19/478). Frequencies of HIV (15.9% (7/44)) and hepatitis C (6.8% (3/44)) were higher in Djoliba village than in other villages. In this area, more than half of volunteers failed to be enrolled. Positives Hbs antigen, HCV and HIV are the most frequent laboratory screening failure reasons, and public health authorities should investigate the high rates of these infections in these study communities.

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THE SNAKEBITE ATLAS: A GROUND-BREAKING, OPEN-ACCESS PORTAL FOR SHARING AND GATHERING GLOBAL, COUNTRY-SPECIFIC INFORMATION ON SNAKEBITE DEATHS, ANTIVENOM AVAILABILITY AND VENOMOUS SNAKES

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Venomous snakebites are medical emergencies that annually kill over 95,000 people residing in some of the most disadvantaged, rural and remote tropical communities, and leave 2-300,000 surviving victims with permanent physical disabilities/disfigurements. Victims require instantly-available First Aid information and rapid access to effective treatment. Attending physicians require immediately-available information as to the most effective, locally-available antivenom. Ministries of Health and medical charities require data and information to plan antivenom-provision and snakebite-management strategies. Acquiring this essential life-saving information is currently laborious, problematic and overly dependent upon personal contact networks. The Snakebite Atlas is a free, open-access resource providing geographically-specific information on the venomous snake fauna (including images aiding snake identification), snakebite incidence, morbidity and mortality data, the brand name and manufacturer details of recommended antivenoms, and links to

recommended First Aid and clinical snakebite-management protocols. The system will also allow data capture, and as such, will be a growing resource. The Snakebite Atlas will comprise a database with Geographic Information System (GIS), a visually rich web interface optimized for mobile, an API for 3rd party integration, and an online/offline smartphone app. The provision of the Atlas as a mobile-optimized website and smartphone app will meet the needs of all users, irrespective of their location. Most rural tropical communities have very limited fixed-line internet access, but good mobile coverage. The online/offline app will allow users to access the information even when mobile coverage is not available, and the app will sync with the central database whenever connectivity is sufficient. The Snakebite Atlas therefore offers a much-needed information-sharing tool for clinical and public health practitioners, biologists and the public.

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RESPONSE TO FEVER AND UTILIZATION OF STANDBY EMERGENCY TREATMENT (SBET) FOR MALARIA IN TRAVELERS TO SOUTHEAST ASIA: A SURVEY-BASED COHORT STUDY

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Guidelines in several European countries recommend standby emergency treatment (SBET) for travellers to regions with low or medium malaria transmission (e.g. Southeast Asia (SEA)) instead of continuous chemoprophylaxis. For this approach travellers are advised to seek for medical assistance within 24 hours in case of onset of fever and to self-administer SBET only if they are not able to consult a doctor within the time period specified. Data on health care seeking behaviour of febrile travellers and utilization of SBET is however scarce as only two studies were performed in the mid-1990s. Since tourism to SEA is constantly increasing and malaria epidemiology has dramatically changed in the meantime more knowledge is urgently needed. For this study 876 travellers to destinations in SEA were recruited in the travel clinic of the University Medical Center Hamburg-Eppendorf. Demographic and travel-related data were collected by using questionnaires, pre-travel advice was carried out and SBET was prescribed in accordance to national guidelines. Post-travel phone interviews were performed to assess health incidents during travel and individual responses of travellers to febrile illness. Out of 714 patients who were monitored, 130 (18.2%) reported onset of fever during travel or 14 days after return. Of those travellers who reported fever, 100 (76.9%) carried SBET during travel. The vast majority of 79 (79%) of febrile travellers did not seek for medical assistance. Overall, 16 (16%) of febrile patients who carried SBET and 6 (20%) of patients who did not carry SBET took the correct measure (doctor visit or SBET intake) as a response to febrile illness, respectively. Only 2 travellers self-administered SBET, but both of them applied the wrong regimen. In view of declining malaria transmission and improving medical infrastructure in most countries of SEA and obvious obstacles concerning SBET as shown in this study we propose to re-evaluate the current strategy. Pre-travel advice for travellers to SEA should focus on the simple message to immediately see a doctor in case of febrile illness.

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MORTALITY AMONG EXTREMELY LOW BIRTH WEIGHT INFANTS IN PERU

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Infant mortality has decreased in recent years given improvements in health care policies all over the world. However, neonatal mortality continues to be a major public health issue, especially among very low birth weight and extremely low birth weight (ELBW) infants. There is no data regarding mortality in ELBW infants in developing countries. The aim of the study was to determine the mortality in ELBW infants and to describe the most common etiologies in three neonatal units in Lima, Peru. We enrolled 77 ELBW (<1000g); 21 with a birth weight of 500-750g and 56 with 751-1000g as part of an ongoing clinical trial in three neonatal units in Lima. All patients were followed until death or discharge. Mean birth weight was 831.9 ± 123g, mean gestational age was 27.4 ± 2.3 weeks and length of hospitalization was 43 days (IQR 13-67). Patients with a birth weight ≤750g had an overall mortality of 85.7% while patients with 751-1000g had an overall mortality of 44.6%. Mortality for infants ≤750g was 23.8% within the first 7 days, 42.9% within the first 14 days and 76.2% within the first 28 days of life. For infants 751-1000g, mortality rates were 8.9%, 21.4% and 28.6% within 7, 14 and 28 days, respectively. Neonatal sepsis was the leading cause of death in both groups (50% for infants ≤750g and 76% for infants 751-1000g). Other causes of deaths included intraventricular hemorrhage, extreme prematurity and respiratory distress syndrome. Mortality was high among ELBW infants, especially in those with a birth weight less than 750g. The greatest risk of neonatal death occurred within the first two weeks of life. More studies focusing on mortality and morbidity in ELBW infants in developing countries are needed. This would help to establish prevention strategies that reduce the risk of sepsis and sepsis-related deaths.

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MANAGING THE FEBRILE CHILD IN THE ERA OF SPREADING ANTIBIOTIC RESISTANCE: DEVELOPMENT OF E-POCT, AN ELECTRONIC ALGORITHM THAT USES HOST BIOMARKER POINT-OF-CARE-TESTS

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The objective was to review the available knowledge on epidemiology, diagnosis, and management of acute infections in children aged 2 to 59 months in the outpatient setting and develop an evidence-based electronic algorithm suitable for resource-poor settings that uses available point-of-care tests to achieve optimal clinical outcome while increasing the rational use of antibiotics. Through a structured literature review in Medline, Embase and the Cochrane Database of Systematic Review targeting outpatients aged 2-59 months, we searched for i) available disease prevalence in resource-poor settings, ii) accuracy of clinical predictors, and iii) performance of point-of-care tests for targeted disease management strategies (biomarkers of inflammation, hemoglobin (Hb), blood sugar, and oximetry). A novel electronic algorithm for the management of childhood illness (e-POCT) was designed based on evidence retrieved. The major

changes compared to IMCI (2014 version) are the following: i) use of vital signs (in particular oxygen saturation and heart rate), Hb and glycemia for classification of severe disease, ii) automated temperature- and age-correction of heartrate and respiratory rate, iii) replacement or elimination of clinical signs with low accuracy, iii) classification of bacterial pneumonia based on a combined respiratory-rate and C-reactive protein cutoff (CRP), iv) antibiotic prescription based on CRP and procalcitonin in fever without source patients, v) use of Hb for depiction of severe disease, vi) increased resources for management of skin infections. This novel smartphone-run algorithm based on new evidence and two point-of-care tests should improve the quality of care of under-fives and lead to more rational use of antibiotics.

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INCREASING PREVALENCE OF HUMAN *DIROFILARIA*SIS IN THE UNITED STATES AND EUROPE

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*Dirofilaria*sis is a mosquito-borne filarial infection of carnivores. Since the zoonotic seroprevalence of dirofilariasis has increased, the objectives of this study were to determine human prevalence rates of dirofilariasis and compare them for significant increases over the period, 1999-2012. Internet search engines identified reports of human dirofilariasis in the United States (US) and Europe with cases defined by positive histopathology. Proportional increases in disease prevalence were stratified by nations and compared for statistically significant differences by chi squares (X2) with significance defined by p-values < 0.05. By 2012, 372 cases of human pulmonary dirofilariasis caused by *Dirofilaria immitis* had been reported worldwide representing a 121% increase since 1999. The greatest increases occurred in Italy and the US with the most significant increase in the US (X2=6.4, p=0.011). By 2012, 1,410 cases of subcutaneous and/or ocular dirofilariasis, caused by *Dirofilaria* repens had been reported worldwide representing a 67% increase since 1999. The greatest increases in cases occurred in Russia (X2=66.4, p <0.0001) and Italy (X2=185.9, p<0.0001). Dirofilariasis is an emerging parasitic disease of dogs and man resulting from a combination of factors including warmer year-round global temperatures with shorter winters extending vector transmission cycles; failing regional mosquito control programs; increasing seroprevalence of *Dirofilaria* infections in wild carnivores; increasing parasite resistance to chemoprophylaxis in domestic dogs with macrocyclic lactones, especially ivermectin; and more frequent international travel to *Dirofilaria*-hyperendemic regions, especially the Southern US, Eastern Russia, and the Italian Piedmont. Residents and vacationers in these high-risk regions need to protect themselves from dirofilariasis with mosquito repellents. More effective chemotherapeutics and improved immunodiagnostics and are needed to control and monitor sentinel animal reservoirs. More frequent regional mosquito control will interrupt prolonged vector-transmission cycles.

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DETERMINATION OF BREAK IN TRANSMISSION IN VECTORS OF LYMPHATIC FILARIASIS GHANA

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Since the implementation of the Global Programme for the Elimination of Lymphatic Filariasis (GPELF) in 2000, MDA has been on-going in many LF endemic countries. Post-MDA monitoring of parasite transmission is to assess the efficacy of MDA, when to stop MDA and for the certification of elimination of the disease. Monitoring of the transmission pattern in the mosquito vectors is as essential as detecting levels of infection the human population, since mosquitoes offer real time estimates of transmission. This study aims to provide evidence of break in transmission in vectors of lymphatic filariasis in some endemic areas of Ghana. A total of 7,072 mosquitoes were collected from 53 communities in two selected districts in Ghana in 2013 and 2014. A total of 4,733 (66.9%) *Anopheles* sp. (comprising of *An. gambiae* s.l, *An. funestus* and *An. pharoensis*), 2,274 (32.2%) *Culex* species and 65 (0.9%) Mansonia species were identified and tested. Mosquitoes were tested in pools of 1 to 20 with an average pool size of 15 using Loop-mediated Isothermal Amplification LAMP). All LAMP positive samples were confirmed with PCR. Three pools (2 *Anopheles* and 1 *Culex*) and 2 pools of *Anopheles* tested positive for W. bancrofti in 2013 and 2014, respectively. Infection rates for *Anopheles* and *Culex* mosquitoes for 2013 were found to be 0.97 and 0.86, while that for 2014 were 1.35 and 0.00, respectively. The average biting rates for both years did not show any significant difference (p > 0.99). Surveillance in humans has shown low levels of microfilariae prevalence but in areas where there is residual infection; xenomonitoring offers real time estimates of transmission in areas where very low levels of microfilaremia may not be detected in humans. Therefore the detection of infection in mosquito vectors is an indication that there may be positive individuals in the area of interest.

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EVALUATION OF ONCHOCERCIASIS TRANSMISSION IN TANZANIA: RESULTS FROM THE TUKUYU FOCUS, 2015

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Onchocerciasis, or river blindness, is the second most prevalent infectious cause of blindness in Sub-Saharan Africa. Tanzania implemented annual ivermectin mass drug administration (MDA) in 2000 in the Tukuyu focus, which had pre-control endemicity as high as 63%. Recent MDA coverage was 69-86%. World Health Organization (WHO) guidelines require Ov-16 antibody positivity to be <0.1% in children at risk before stopping MDA. This study compared diagnostic tests in the Tukuyu focus in relation to the guidelines and examined age-group specific differences in prevalence. Eleven villages near vector breeding sites were selected, and an age-stratified random sample was performed. Participants underwent a questionnaire, skin examination, skin snips for microfilaria count and PCR (≥5 years-old only), and blood draw for: rapid Ov-16 antibody test (RDT), Ov-16 ELISA, immunochromatograph (ICT) filariasis rapid test, and daytime blood smears. A total of 695 households (HH) were selected; 617 (98%) HH participated yielding 948 individual participants. A total of 499 (52.7%) participants were male; the median age was 12 years (interquartile range: 6-26 years). Past-year ivermectin use was reported by 522 (65.7%) of eligible participants; itchy skin was reported by 207 (21.9%). Only 12 (1.3%) participants had nodules; manifestations of onchodermatitis occurred in <1% of participants. All skin snips, ICT card tests, and daytime blood smears were negative; 38 (5.5%) participants had a positive RDT. Weighted, adjusted age group specific prevalence was: 0-5 years, 0.5%; 6-10 years, 0.4%; 11-15 years, 0.8%; 16-20 years, 2.2%; >20 years, 10.5%. The low burden of symptomatic disease and few positive RDTs demonstrates that annual MDA has had a significant impact on the disease in the Tukuyu focus. However, the Ov-16 positivity in children failed to meet the WHO guidelines for stopping MDA. Results of skin snip PCR, OV-16 ELISA, and a simultaneous vector collection will

add to the data presented here. Further consideration is needed to assess if the 0.1% threshold is too strict and if extended age groups (≥10 years-old) should be included in future assessments.

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NEW METHODS TO MEASURE CHANGES IN INFECTIOUS DISEASE TRANSMISSION FROM QUANTITATIVE ANTIBODY MEASUREMENTS: EXAMPLES USING *WUCHERERIA BANCROFTI*, *PLASMODIUM FALCIPARUM* AND ENTERIC PATHOGENS

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Global elimination efforts for neglected tropical diseases and malaria rely on accurate estimates of pathogen transmission intensity to target control programs and evaluate their effectiveness. Serological antibody levels have proven to be a sensitive marker of pathogen exposure, and advances in multiplex serological assays have created enormous potential for large-scale, integrated infectious disease surveillance. We developed a novel, nonparametric method using recent advances in ensemble machine learning and statistical estimation to measure changes in transmission from quantitative antibody levels that can be applied to diverse pathogens of global importance. We compared age-dependent immunoglobulin G curves in intervention (Nigeria, Cook Islands) and observational (Haiti, United States) settings with differences in transmission intensity for multiple pathogens, including: lymphatic filariasis (*Wuchereria bancrofti*), malaria (*Plasmodium falciparum*), enteric protozoans (*Cryptosporidium parvum, Giardia intestinalis, Entamoeba histolytica*), enteric bacteria (enterotoxigenic *Escherichia coli, Salmonella* spp.), and norovirus groups I and II. Age-dependent antibody curves followed a characteristic shape across pathogens that aligned with predictions from basic mechanisms of humoral immunity. Changes in pathogen transmission led to shifts in fitted antibody curves that were remarkably consistent across pathogens, assays, and populations. Summary differences between curves provided a robust and sensitive measure of changes in transmission, with greatest sensitivity among young children. Summary *P. falciparum* antibody measures correlated strongly with the entomological inoculation rate (Spearman's $\rho = 0.75$). We will illustrate easy to use, open source software to implement the approach. The method generalizes to pathogens that can be measured in high-throughput, multiplex serological assays, and scales to estimation problems requiring high spatiotemporal resolution -- features that make the approach well-suited to integrated surveillance of global infectious disease elimination efforts.

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THE FIRST SUCCESSFUL CONFIRMED ELIMINATION OF AN ONCHOCERCIASIS FOCUS IN AFRICA: ABU HAMED, SUDAN

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Mass treatment with ivermectin for onchocerciasis was stopped in 2012 in Abu Hamed, an isolated focus on the River Nile in northern Sudan. A three year post-treatment surveillance (PTS) ensued, at the end of which an evaluation was conducted in 2015 following the current WHO guidelines for verification of onchocerciasis elimination. Vector black flies were collected from sentinel breeding sites and fingerprick bloodspots were collected from children ≤ 10 years old resident in 35 communities within the focus. O-150 PCR screening of 19,191 flies from 4 sites found no flies carrying *Onchocerca volvulus* larvae (0%, 95% Upper Confidence Limit = 0.16), and serological testing of 5266 children identified only one Ov16 seropositive child (0.019%, 95% UCL = 0.074); who was negative when screened by O-150 PCR assay. These results indicate that for the first time in Africa, onchocerciasis elimination has been verified following a successful PTS in Abu Hamed.

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USABILITY, ACCEPTABILITY, AND IMPLICATIONS OF UTILIZING THE SD BIOLINE ONCHOCERCIASIS IGG4 RAPID TEST IN ONCHOCERCIASIS SURVEILLANCE IN SENEGAL

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Global efforts toward control and elimination of onchocerciasis have made significant progress through community-directed treatment with ivermectin (CDTI). After years of successful CDTIs, control programs need to determine whether *Onchocerca volvulus* transmission has been interrupted and therefore treatment can be stopped. In many communities, the current testing method is skin snip microscopy; however, this method is less acceptable and has declining sensitivity in low-prevalence settings. The 2016 World Health Organization "Guidelines for Stopping Mass Drug Administration (MDA) and Verifying Elimination of Human Onchocerciasis" recommend using Ov16 serology tests, in addition to entomological evaluations, for post-treatment and post-elimination surveillance. The SD BIOLINE Onchocerciasis IgG4 rapid test (Standard Diagnostics, South Korea) is a rapid Ov16 serological diagnostic test (Ov16 RDT) that is field friendly, noninvasive, simple to use, and low cost. Our hypothesis is that the Ov16 RDT is an improved tool over skin snip microscopy for country programs to monitor their progress toward stopping MDA and reaching elimination. The study incorporates the Ov16 RDT in ongoing onchocerciasis surveillance activities in Senegal in 15 villages among 1,250 individuals over the age of 5 years. The study compares age-prevalence curves, workflow, and cost analyses of the Ov16 RDT and skin snip microscopy. Additionally, usability and community acceptance of the Ov16 RDT are assessed using a mixed methods research design, and a mobile phone-based data collection tool is piloted along with traditional data collection systems. This study provides key evidence of the feasibility of implementing the Ov16 RDT to make critical decisions about whether to continue, halt, or re-initiate MDA efforts in communities. The results of this study may be informative to other country programs interested in adopting this new tool, particularly as these country programs quickly move from control to elimination of onchocerciasis.

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THE FEASIBILITY OF A 'RE-MAPPING' PROTOCOL FOR LYMPHATIC FILARIASIS IN AREAS WHERE TRANSMISSION IS UNCERTAIN IN ETHIOPIA

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Lymphatic filariasis (LF) is one of the world's leading causes of permanent disability. WHO proposed a comprehensive strategy to eliminate LF by 2020, interruption of transmission through MDA and morbidity management. The global program to eliminate lymphatic filariasis (GPELF) recommends mapping as an initial step to determine the need of MDA in Implementation units (IUs). The existing WHO guideline recommends two sites per IU should be selected for mapping and a sample of 100 adults should be tested for antigenaemia. In low transmission setting this strategy has limitations. An alternative complementary mapping method that could resolve this situation is useful. This study was, therefore, designed to assess the Lf endemicity level of IUs in Ethiopia which have low transmission setting. The 2013 mapping in Ethiopia resulted in 45 IUs with antigenemia result not enough for programmatic decision making. To solve this gap the school based re-mapping study was conducted in two phases; phase I was conducted last year in 8 IUs, to evaluate the protocol itself. The study proceeded to Phase II after the result of the phase I re-mapping was analyzed and interpreted. In this survey, schools were selected by either systematic or cluster sampling, based on the number of schools in the IUs. From each selected school children of grade 4 to 8 were sampled systematically and tested for antigenemia. The number of positive result was compared against the critical value. From 41 IUs involved in second phase re-mapping survey, antigenesimia was tested from 16,365 children of target grades in selected schools. In 39 of the IUs, the number of antigen positive identified in the re-mapping surveys was below the critical cut off, suggesting transmission of LF is not ongoing in these IUs. Where as in two of the IUs, the number of antigen positive identified was above the critical value suggesting that the LF transmission is ongoing. In low prevalence areas, where the current WHO protocol has several limitations, this re-mapping study design will provide enough information on the LF transmission situation which may help programs to make evidence based decision.

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LONGITUDINAL EVALUATION OF ONCHOCERCIASIS 2012-2015 IN THE MID NORTH FOCUS IN UGANDA

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Onchocerciasis is a neglected tropical disease that is targeted for elimination. Uganda implemented annual mass drug administration (MDA) with ivermectin (IVM) in this focus in 2009 and began twice yearly MDA in October 2012. Late in 2012 it also implemented a 1-year vector control program. We conducted an initial survey of 500 people in September 2012 and a follow-up survey in September 2015. All participants underwent questionnaires, skin examination for *Onchocerca*-associated lesions, two skin snips and a venous blood draw. Skin snip microscopy, PCR and OV-16 ELISA were performed on the collected specimens. A total of 343 (68%) people re-enrolled in the 2015 study; 209 (60%) were female, and the median age was 36 years (range 10-90 years). The use of IVM was high at both time points (86% vs. 88%). Comparisons between 2012 and 2015 showed that significantly fewer people at follow up had skin nodules (54% vs 24%), positive skin snip results (25% vs 6%), as well as skin lesions: acute papular onchodermatitis (3% vs. 0.3%), chronic papular onchodermatitis (6.5% vs 0.6%), lichenified onchodermatitis (6% vs 0%), and skin depigmentation (9.7% vs 1.6%) (McNemar's test, $p<0.05$). Significant reductions in microfilarial density in skin snips (2.3 vs 0.2 microfilariae/2 snips/person) and OV-16 ELISA optical density (OD) values (2.08 [SD=1.58] vs 0.92 [SD=1.14]) (paired t-test, $p<0.05$) were also observed; however, the prevalence of OV-16 positivity did not change (85% vs 79%). Taken together, these results show that a significant reduction in disease morbidity occurred in North Uganda after three years of twice per year IVM MDA and one year of vector control. Both skin snip microscopy and quantitative OV-16 ELISA provided important information on MDA program impact. Correlating OV-16 ELISA results to other markers of infection might allow for the differentiation of on-going versus suppressed transmission. Furthermore, the decline in OV-16 ELISA values suggest its potential use as an alternative to skin snip PCR when assessing those few individuals with OV-16 positive results in program evaluations to stop MDA.

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DETECTION AND EVALUATION OF ANTI-OV-16 ANTIBODIES FOR ONCHOCERCIASIS SURVEILLANCE IN THE CENTRAL ENDEMIC ZONE OF GUATEMALA

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Current WHO guidelines recommend testing for OV-16 serologic responses in children to determine when it is appropriate to stop mass drug administration (MDA) for onchocerciasis. Understanding the decrease in antigen-specific seropositivity over time might allow the inclusion of older age groups for these assessments or post-treatment surveillance (PTS). In the Central Endemic Zone (CEZ) of Guatemala, interruption of onchocerciasis transmission was observed in 2011 and confirmed in 2014. A follow-up study was conducted in October 2014 to assess the serological response to onchocerciasis among people tested between 2003 and 2009, while transmission was ongoing. Comparisons were based on a normalized OV-16 ELISA, with a positive cutoff of 40 Activity Units (AU). Results from participants with positive serology at baseline were used to conduct a preliminary estimation of temporal antibody decay rates using a mixed effects linear regression model. A total of 230 people (120 female, 52%) were enrolled, 85 with previous positive OV-16 ELISA results and 145 who were negative. The 85 positives contributed 93 retrospective data points with mean AUs of 86 (n=11), 174 (n=59), 108 (n=19) and 82 (n=4) in years 2003, 2006, 2007 and 2009, respectively, and 40 [95% CI 23.8-55.8] in the 2014 follow up. Those considered negative contributed 155 data points, with mean AUs of 30 (n=18), 19 (n=59), 10 (n=67) and 0.1 (n=11) in 2003, 2006, 2007 and 2009, respectively, and 6 [95% CI 3.9-7.7] in the 2014 follow up. Additionally, none of the 77 study participants under the age of 20 years had a positive serologic result. Temporal analysis of AUs showed a mean decay rate of 0.31/year (SD= 0.20) and an average half-life of 2.25 years. These results concur with previous assessments that onchocerciasis transmission had been interrupted in the CEZ. The antibody responses to OV-16 appear to have decreased over time, presumably due to the lack of exposure to viable adult worms. These findings also highlight the importance of conducting further studies to determine if people ≥10 years of age could be included in evaluations for stopping MDA, PTS, or post-elimination surveillance.

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A DELPHI CONSULTATION TO ASSESS INDICATORS OF READINESS TO PROVIDE QUALITY HEALTH FACILITY-BASED LYMPHEDEMA MANAGEMENT SERVICES

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The World Health Organization (WHO) in collaboration with partners is developing a toolkit with resources to guide lymphatic filariasis (LF) morbidity management and disability prevention (MMDP) implementation and evaluation. Assessment of the readiness of programs to provide quality lymphedema management services is recommended via a direct facility inspection. As part of tool development, a Delphi consultation was implemented to gain consensus on the proposed themes and tracer indicators to measure readiness to provide quality health facility-based lymphedema management services. A seven-point Likert-type scale was used to rank the importance of proposed themes and tracer indicators. Consensus for inclusion of the indicator was defined a priori as 70% or more of respondents ranking the proposed indicator in the top three points (5-7). Purposive sampling was used to select 43 representative experts including neglected tropical disease (NTDs) country representatives, program implementers, and technical experts. A 55.8% response rate (n=24) was achieved for the first round of the survey. The majority of respondents had ten or more years of expertise (n=17, 70.8%) in MMDP or NTDs. Analysis of the first round of data demonstrated that consensus for inclusion had been reached across all proposed indicators including trained staff (mean=6.9, standard deviation (SD)=0.34), case management and education materials (mean=6.1, SD=0.65), water infrastructure (mean=6.3, SD=0.81), medications and commodities (mean=6.3, SD=0.69), patient tracking system (mean=6.3, SD=0.85), and staff knowledge (mean=6.5, SD=6.5, SD=0.66). The results from this analysis were used to inform revisions of the direct inspection which will be piloted in several countries to further refine tools to assess readiness to provide quality lymphedema management services.

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REEXAMINATION OF AREAS WITH PERSISTENT LYMPHATIC FILARIASIS 9 YEARS AFTER CESSATION OF MASS DRUG ADMINISTRATION IN SRI LANKA

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Sri Lanka was one of the first countries to initiate a lymphatic filariasis (LF) elimination program based on WHO guidelines. Following several years of mass drug administration (MDA) with DEC alone, the Anti-Filariasis Campaign (AFC) provided 5 annual rounds of MDA with DEC plus albendazole in all endemic districts in the country from 2002-2006. The AFC and other groups have conducted extensive surveillance activities since 2006. Microfilaremia (Mf) rates have been consistently <1% in all sentinel and spot check sites since that time, and all 11 evaluation units passed school-based transmission assessment surveys (TAS) in 2013. We have previously reported results from comprehensive surveillance studies conducted in 2011-2013 that documented low-level persistence of LF in 19 high risk Public Health Inspector areas (PHI, mean population 25,000) spread across 8 endemic districts. We now present results from repeat comprehensive surveys conducted in 2015 (8 to 9 years after the last round of MDA) in 5 PHI areas that had the strongest LF signals in the prior study. These surveys assessed community CFA and Mf rates, CFA and anti-filarial antibody rates in school children (ages 6-8), and filarial DNA rates in *Culex quinquefasciatus* collected with gravid traps. Two areas had encouraging results with improved LF parameters (Pw and KN), but three other areas (Uw, Amb, Wg) showed no significant change. Our study also identified a new hotspot in Galle district with alarmingly high LF parameters (community CFA 3%, Mf 1%, anti-Bm14 antibodies in school children 5.7%, and a filarial DNA rate of 5.2% in the vector). Thus areas with persistent LF in Sri Lanka appear to be close to the transmission breakpoint; LF is likely to disappear without further intervention in most areas, while other areas may require more work. We think that LF elimination programs should consider using methods other than school-based TAS to identify areas with persistent LF following completion of MDA. In addition, long term surveillance may be needed to verify that LF has been eliminated, and this may be especially true in areas like Sri Lanka where the parasites are transmitted by *Culex* mosquitoes.

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DEFINING AND DETECTING SUBOPTIMAL RESPONSES TO IVERMECTIN IN PATIENTS WITH ONCHOCERCIASIS

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Mass drug administration (MDA) with ivermectin is the cornerstone of global efforts to eliminate onchocerciasis (river blindness). Several epidemiological studies in Ghana and Cameroon have reported so-called suboptimal or atypical responses to ivermectin in patients infected with *Onchocerca volvulus*, the causative filarial parasite of onchocerciasis. These responses are characterised by a faster than expected rate of skin repopulation by parasite microfilariae following treatment and have been interpreted as warning signs of decreased efficacy of the anti-fecundity effects of ivermectin on adult female worms, potentially caused by emerging (reproductive) resistance. Yet, there is currently no clear and objective definition of what constitutes a suboptimal response nor any reliable method for detangling the true underlying drug response from the high degree of statistical sampling error incurred when counting microfilariae in skin snips. Here we tackle this problem using an individual-based statistical model which we fit in a Bayesian framework to individual patient data from the first set of clinical trials of ivermectin against human onchocerciasis conducted in the mid 1980s, before the widespread use of ivermectin for MDA. We use the fitted model to define predictive distributions of typical responses to ivermectin in drug-naïve patients and validate these using both censored data from the same set of (historical) trials and contemporary data from ivermectin-treated control patients who participated in the recent phase II clinical trial of moxidectin. We discuss how the analytical framework can be used to identify and monitor suboptimal responses to ivermectin in populations undergoing long-term MDA.

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THE EFFICACY OF PREVENTATIVE CHEMOTHERAPY DRUGS FOR THE TREATMENT OF LYMPHATIC FILARIASIS: A SYSTEMATIC REVIEW AND MODEL-BASED META-ANALYSIS

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The principal intervention strategy to control and eliminate lymphatic filariasis (LF) is so-called preventative chemotherapy (PCT) by mass drug administration (MDA) with combinations of albendazole and either ivermectin or diethylcarbamazine. These therapies, although of uncertain

efficacy against adult parasites (macrofilariae), suppress numbers of blood-borne microfilariae for approximately one year after treatment. Because microfilariae are infectious to the mosquito vectors of lymphatic filariae, if enough infected people are treated, transmission between treatment rounds will be markedly reduced or interrupted completely. Local elimination is possible if this can be maintained for at least as long as the natural life-span of the macrofilariae. While compelling, a key uncertainty in this elimination algorithm, is for how long following treatment microfilariae are sufficiently suppressed such that individuals adhering to MDA are essentially removed as contributors to transmission. Indeed, the efficacy of PCT drugs is currently incorporated into LF transmission models (that are used to predict timeframes for elimination) with considerable uncertainty, based on expert opinion and semi quantitative ultra-sonographic data on macrofilariae. Here, we present a literature review to identify clinical and field trials of the efficacy of the PCT drugs used to treat LF and extract relevant data on numbers of microfilariae at different times following treatment. We develop a simple mathematical model to describe the population dynamics of microfilariae following treatment and fit this to the available data. We use the fitted model to characterise the efficacy of PCT combinations against LF and discuss the application of our results to mathematical transmission models of LF and to the detection of suboptimal or atypical drug responses.

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IMPACT OF IVERMECTIN MASS TREATMENT ON THE BURDEN OF *ONCHOCERCA*L SKIN AND EYE DISEASE: DETAILED MODEL PREDICTIONS UP TO 2025

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The African Programme for Onchocerciasis Control (APOC) coordinated from 1995-2015 annual ivermectin mass treatment to control morbidity of onchocerciasis (river blindness). These efforts should be maintained even after 2015 to achieve elimination in 80% of all endemic countries by 2025, although this may not be achievable everywhere due to late onset of infection and/or low treatment coverage. Even if elimination is realised, a considerable chronic disease burden remains due to earlier infection. To understand the need for alternative interventions and new drugs, we predict trends in infection and onchocercal morbidity over time up to 2025, stratified by age and sex. We use the individual-based model ONCHOSIM to predict trends for a range of scenarios, varying by type of onchocerciasis (forest / savannah), baseline endemicity, history and future of control. The model was extended to make detailed predictions for a wide spectrum of forms of onchocercal skin disease, which so far were considered to only include itch. Model parameters were quantified to reproduce association between infection and morbidity using data from a TDR-funded multi-country field study on onchocercal skin disease as well as literature on visual impairment and blindness. The prevalence of infection, morbidity and excess mortality decline progressively over time up to 2025. The prevalence of reversible skin disease (e.g. troublesome itch, acute and chronic papular, and lichenified onchodermatitis) declines rapidly with waning infection prevalence, with the rate of the decline depending on achieved therapeutic coverage. Irreversible manifestations i.e. visual impairment, blindness, atrophy, depigmentation, and hanging groin decline much more gradually. This study provides better insight in expected trends of infection and onchocercal morbidity. We discuss expected trends in disease burden for endemic countries and Africa as a whole, which will be useful to policy makers and national onchocerciasis control programmes.

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APPLYING A MOBILE SURVEY TOOL FOR ASSESSING LYMPHATIC FILARIASIS MORBIDITY IN MTWARA MUNICIPAL COUNCIL OF TANZANIA

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A number of methods have been used to estimate lymphatic filariasis (LF) morbidity, including: routine programmatic data, cluster random surveys and the "town crier" method. Currently, few accurate data exist on the global LF morbidity burden. We aimed to estimate prevalence of lymphedema and hydrocele in Mtwara Municipal Council using mobile phone based survey. A cross-sectional survey was conducted among adults of Mtwara municipal council with access to mobile phones. A sample size of at least 384 completed surveys was required to estimate prevalence of lymphedema (both males and females) and hydrocele (males only) morbidity of 50% within a 5% error margin given a 5% level of significance and 95% confidence level. Eligible mobile phone users received a short message text (SMS) requesting consent to participate in the survey. A total of 10 questions were administered via interactive SMS through GeoPoll, a survey platform developed by Mobile Accord (research.geopoll.com). The survey was completed over a period of 4 days. A total of 8,759 surveys were sent to mobile phone subscribers of whom 1,330 (15.2%) opted-in to complete the survey. A total of 492 (37.0% or those opted-in, 384 male and 108 female) people completed the survey. Lymphedema and hydrocele signs were reported by 20.9%; (95% confidence interval [CI] 17.4 - 24.8) and 20.6%; (95% CI 16.6-25.0) of respondents, respectively. Majority of hydrocele patients (59.5%) and 46.6% of lymphedema patients reported having sought treatment. The proportion of patients reporting similar symptoms among friends are relatives was 35.8% and 70.9% for lymphedema and hydrocele, respectively. The findings suggest that mobile phone based surveys are a practical and rapid approach of undertaking morbidity surveys. While further methods of clinical examination are needed to verify the findings, this approach can be expected to encourage identification of lymphedema and hydrocele morbidity at community level and provide evidence where morbidity management services are warranted.

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STEPS TOWARDS ELIMINATION: RE-EVALUATION OF LYMPHATIC FILARIASIS PREVALENCE IN TANZANIA

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Despite positive strides made in the control of lymphatic filariasis (LF), this disease continues to cause morbidity and mortality in Tanzania. In order to inform targeted and effective mass drug administration (MDA) in the country, the national neglected diseases program (NTDCP) conducted remapping in 2015. The purpose of the re-mapping surveys was to to assess LF prevalence levels and to identify new LF transmission hotspots in areas where MDA had not been implemented before. The 2015 LF remapping exercise was conducted in nine regions covering 63 districts in the Lake, Western and Northern zones in Tanzania, with support from USAID through the ENVISION Project and from the Task Force for Global Health. The 9 regions included Geita, Simiyu, Mwanza, Arusha, Shinyanga,

Mara, Kagera, Kigoma and Kilimanjaro. This was a randomized 30-cluster school survey design, in which 30 primary schools were randomly selected from each district. Fifteen children aged from ≥10 years were selected randomly from each school. A total of 1,770 primary schools were randomly sampled and 29,054 students aged ≥10yrs old were tested for LF. Each child was tested using the immunochromatographic card test (ICT) for LF. Of these, six positive LF tests were confirmed, equivalent to 0.021% of the total number of students tested. The confirmed positive LF tests were found in Magu DC, Ukerewe DC, Misenyi DC, Moshi DC and Bariadi TC. The results of the re-mapping indicated that these districts do not require MDA. This is an important step in scaling down MDA interventions in Tanzania and now the NTD program will focus on the remaining districts to achieve elimination.

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MOSQUITO BITE HETEROGENEITY INFLUENCES LYMPHATIC FILARIASIS PREVALENCE, INTENSITY AND OPPORTUNITIES FOR CONTROL

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Global efforts to eliminate lymphatic filariasis have achieved many successes in the local elimination of this debilitating neglected tropical disease. An emerging challenge to the elimination program are heterogeneities in village-specific characteristics within district-level interventions. One important question is how aggregated exposure to infective bites and aggregated worm burden impact the choice of intervention and the optimum coverage needed to achieve elimination and how this varies spatially. We test the hypothesis that heterogeneous disease patterns are derived primarily from the distribution of infective bites received by an individual over their lifetime. We also test the hypothesis that vector control results in more heterogeneous exposure which may influence the time to local elimination. We employed a dataset from five villages in Papua New Guinea characterized by moderate to high transmission of LF. These included spatially resolved anopheline densities surrounding the bednet distribution, and individual antigenemia and microfilaremia. We calculated the heterogeneity of biting density and mf by the fitting of a negative binomial distribution at both village-level and at the level of individuals. This was complimented with a full geospatial model of filariasis infection based on the underlying distribution of bites. We found that the heterogeneity of bites at the village level is a very poor indicator of heterogeneity in the mf count (correlation less than 0.1). We observed a significant increase in bite risk heterogeneity, however the decrease in biting density achieved by bednets more than offset the increase in bite aggregation, resulting in a shorter recommended course of mass drug administration. At the individual level, the biting density was a stronger indicator of mf burden, although there was a significant variation in the distribution unaccounted for. This highlights the need for further investigation on how the population-level distribution of parasites arises from environmental, geographic and individual factors.

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ONGOING TRANSMISSION OF ONCHOCERCIASIS IN THREE COMMUNITIES IN MASSANGAM DISTRICT IN WEST CAMERON AFTER 18 YEARS OF MDA OF IVERMECTIN

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The West Region of Cameroon has been under annual ivermectin mass distribution (MDA) for more than 15 years but transmission is on-going. High *Onchocerca volvulus (O.V)* infections have been identified through studies conducted by Sightsavers in collaboration with the Ministry of health of Cameroon, in the Massangam health district and Makoupsap village in particular. There was therefore a need to clearly delineate the boundaries of this potential high transmission focus. We conducted epidemiological and entomological surveys to evaluate the prevalence of Onchocerciasis in the communities around Makoupsap, map out the breeding sites of *Simulium damnosum* s.l. and thus delineate the boundaries of such a potential focus. Nodule prevalence in communities within 20 Km of Makoupsap were determined through clinical examination and prevalence of *OV* microfilaria was determined in children up to 10 years using the IgG_4 (Ov 16) and positive children to OV-16 were skin sniped . Breeding sites were prospected for blackfly larvae and adult blackflies were collected on Esperanza Window traps using hand-held battery powered aspirators. In total 2375 persons participated in the study. The nodule prevalence was 3.4% with children of 6-10 years having nodules in two communities. 898 persons were skin snipped and 115 were positive giving a microfilaria rate of 12.9%. 1530 children were assessed using OV-16 and 148 were positive giving a prevalence of 1.5%. 4598 blackflies were caught during the 9 days of collection. Morphological identification revealed that *S. squamosum s.s.* was the only vector in this region. 3162 females were dissected. Of these, 633 (20.02%) were parous, while 8 (0.25%) were infected and 2 (0.06%) were infective. Mbam River is probably the most important source of biting *S. squamosum s.s.* during the high biting season. These surveys showed an on-going transmission of onchocerciasis in 3 communities despite 18 years of Ivermectin MDA.

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IMPLEMENTATION OF A FACILITY-BASED INSPECTION TOOL TO ASSESS QUALITY OF LYMPHEDEMA MANAGEMENT SERVICES IN VIETNAM

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Lymphedema (LE) management activities in Vietnam were initiated in 2004, when staff were trained to provide facility-based services for patients with LE. With the support of USAID's MMDP Project, Vietnam piloted a WHO-developed direct inspection tool to measure the quality of health facility-based LE management services. A list of all commune health stations (CHS) in areas with known LE patients was compiled. Patients were reported from 213 CHS in the Northern region, 2 CHS in Central region, and 2 CHS in Southern region. About 10% of facilities in the north were randomly selected, whereas all facilities with patients were selected in the Central and Southern regions. A standardized questionnaire was administered at each facility capturing data on 14 equally-weighted tracer

indicators across six quality themes: trained staff, case management and education materials, water infrastructure, medications and commodities, patient tracking system, and staff knowledge; as well as patient knowledge. Scores were generated for the 32 CHS surveyed, as well as national scores for each tracer indicator. The average facility score was 8.8 out of 14.0 possible points (62.9%), and ranged from 4.0 (28.6%) to 13.0 (92.9%). The national average score for each of the tracer indicators was: staff trained in last two years (0.0%); availability of LE management guidelines (56.3%), availability of information, education, and communication materials (15.6%); reliable improved water infrastructure (93.8%); availability of antiseptics (81.3%), antifungals (43.8%), analgesics or anti-inflammatories (96.9%), oral/injectable antibiotics (93.8%), supplies for LE and acute attack management (100.0%); LE patients recorded in last 12 months (9.4%); staff knowledge about LE signs/symptoms (62.5%); staff knowledge about LE management strategies (71.9%); staff knowledge about signs/symptoms of acute attacks (81.3%); and staff knowledge about acute attack management strategies (75.0%). Attrition of trained health staff and depletion of education materials are some areas where Vietnam is planning to reinforce to ensure quality LE services.

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ASSESSING AN IMPORTANT BARRIER TO ONCHOCERCIASIS ELIMINATION: DETERMINANTS AND CHARACTERISTICS OF *LOA LOA* INFECTION AND INTENSITY IN CAMEROON AND GABON

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As the 2020 deadline for elimination of onchocerciasis approaches, it is essential to determine the mapping and treatment needs of areas with untreated hypo-endemic onchocerciasis (<40% prevalence) currently ineligible for mass drug administration (MDA) with ivermectin due to co-endemicity of *Loa loa* infection. Intensity of *L. loa* infection is associated with likelihood and severity of serious adverse events (SAEs) to ivermectin MDA. To inform guidelines for future testing and treatment decisions in 10 *L. loa* endemic countries, we used existing *L. loa* prevalence and intensity data from Gabon (10214 individuals tested in 2014-2015) and Cameroon (5574 individuals tested in 2012-2013) to describe demographic predictors of *L. loa* infection. We used the following guidelines to characterize intensity of *L. loa* infection: no infection detected (0 mf/mL), low intensity (<8,000 mf/mL), medium intensity (8,000-<30,000 mf/mL), and high intensity (>=30,000 mf/mL). In Gabon, 8355 (82%) had no infection, 1503 (15%) had low-intensity infection, 265 (3%) had medium-intensity infection, and 91 (1%) had high-intensity infection. In Cameroon, 3929 (70%) had no infection, 1346 (24%) had low-intensity infection, 215 (4%) had medium-intensity infection, and 44 (2%) had high-intensity infection. Males had a higher prevalence of infection in both Gabon (21% vs. 16%, p<0.0001) and Cameroon (34% vs. 25%, p<0.0001). In both countries, *L. loa* prevalence increased with age. In Gabon, 5% of individuals under age 15 were infected compared to 26% of individuals ages 60 and older. In Cameroon, 13% of individuals under age 15 were infected compared to 37% of individuals 60 and older. High intensity infections were rare but found in all age groups in both countries. These results suggest that *L. loa* testing targeting males and older individuals would be most likely to detect *L. loa* infection in a community.

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LOSS OF CROSS-REACTIVE FILARIAL ANTIGENEMIA IN PERSONS WITH LOIASIS IN CENTRAL CAMEROON

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Recent reports have shown that some persons with loiasis have positive *Wuchereria bancrofti* antigen tests, and this problem is more common in persons with high *Loa loa* microfilaria (Mf) counts. False-positive antigen tests present a challenge for mapping and monitoring of LF elimination efforts in central Africa. We collected blood samples from adult volunteers in *L. loa*-endemic areas in central Cameroon to obtain material for research on cross-reactive filarial antigen(s). We collected blood samples from 183 persons living in the Akonolinga and Awae health districts. Eighty-nine (49%) of these participants had tested positive for loiasis in prior studies, and 18 (10%) had tested positive for *W. bancrofti* antigenemia by ICT in 2013 or 2015. None of the new blood samples ICT positive, and only 3 of 183 plasma samples from the same participants were weakly positive by Alere FTS. *L. loa* Mf counts in the 18 persons previously ICT positive were slightly higher during the present study (median 17, 540, IQR 12,120 - 36,620) than in the prior studies (median 11,870, IQR 5,240 - 32,540). Loa Mf counts were lower among participants with previously negative ICT results (median 2,000, IQR 340 - 4,480), and comparable to their counts in the prior studies (median 2,720, IQR 840 - 6,900). No participant had *W. bancrofti* Mf in night blood smears. These results suggest that persons with loiasis may spontaneously clear a cross-reactive filarial antigen detected by ICT card tests and that this is independent of their clearance of Mf. Our results may also help to explain why many areas in Africa with loiasis do not report falsely positive filarial antigen tests. This variability may be related to differences in infection intensity and/or variable clearance of filarial antigenemia in different regions or seasons.

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PREVALENCE OF ANTIBODIES TO WB123 SIX YEARS AFTER ELIMINATION OF LYMPHATIC FILARIASIS AS PUBLIC HEALTH PROBLEM IN TOGO

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Togo has successfully eliminated lymphatic filariasis (LF) as a public health problem. The last mass drug administration (MDA) targeting LF was in 2009. Since then, LF surveillance has included lab-based screening of nighttime blood films for microfilaria in patients tested for malaria and regular testing of a convenience sample of persons across Togo using the Binax Now® ICT, with follow-up blood films in those who test positive. Togo has also passed two post-MDA transmission assessment surveys. During six years of surveillance, only 8 individuals have tested positive for active infection, and only 3 of those were residents of Togo. In 2015, Togo employed the InBios Filaria Detect IgG4 ELISA to establish the prevalence of antibodies to Wb123 six years after interrupting LF transmission and to assess the utility of the Wb123 ELISA for LF surveillance. The survey was integrated with an integrated NTD impact assessment. At two schools in all 157 peripheral health units of the 8 districts previously endemic for lymphatic filariasis, a convenience sample of 8 school-going children age 6 to 9 years old provided blood spots on filter paper (DBS) for testing by Wb123 ELISA. DBS were obtained from 2344 children. Wb123 ELISA testing is ongoing. All samples run to date appear to be negative for Wb123 antibodies but interpretation of test results is challenging; negative and positive cut-off values are not established and results are

best interpreted using the expectation maximization algorithm (EMA). ELISA directions recommend testing 100 confirmed LF-positive samples, 100 known LF-negative samples, and 100 samples from individuals with other known filarial infections or non-filarial febrile illness. This proves challenging in the setting of LF elimination, and limits utility of the test. Once all samples have been run, the EMA should yield interpretable results. The Wb123 ELISA may be useful for tracking antibody prevalence over time as a means of surveillance for resurgence of LF in Togo, but the need for skilled technicians and difficulty with interpretation pose challenges.

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A BIOINFORMATICS APPROACH TO ASSESS PARASITE KINASES AS DRUG TARGETS USING ANTI-CANCER DRUGS

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The role of human kinases in carcinogenesis has been largely demonstrated. Such knowledge has allowed the development of a variety of kinase inhibitors that target mutated kinases in their kinase domains. At the contrary, protein kinases expressed by parasites have been less studied and their participation in infection remains poorly understood. The present work aimed to determine similarities and differences, at sequence and structure levels, in a set of human 21 kinases and their orthologs from 9 parasites. Microorganisms were divided in carcinogenic parasites (i.e. *Schistosoma hematobium, Ophistorchis viverrini, Clonorchis sinensis*) and non-carcinogenic parasites (i.e. *Plasmodium falciparum*, *Taenia solium, Trypanosoma cruzi, Fasciola hepatica, Leishmania donovani,* and *Echinococcus granulosus*). By applying sequence alignment, phylogeny algorithms and 3D-structure analysis, the comparison of human kinases and their counterparts in parasites was established. Human cancer-related kinases had homologs in parasites, with up to 69% amino acid sequence identity. Such level of conservation was considerably high given the large phylogenic distances among the species analyzed. The human cytosolic kinases showed higher amino acid sequence conservation than human membrane kinases when compared to their homologs in parasites. The cytosolic kinases BRAF, AKT-1, ABL-1, c-SRC, and CDK8 had the higher level of conservation and were present in carcinogenic and non-carcinogenic organisms. A coevolution of kinases in both the host and the parasite is plausible to explain the highly conservation observed among kinases. Residues involved in binding of inhibitors to the active site were inspected in human BRAF, AKT-1, ABL-1, c-SRC, CDK8 and found that most of them were conserved in the corresponding sequences of parasite orthologs. This observation can be exploited and taken as an advantage to repurposing human kinases inhibitors into their homologues in parasites.

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DEVELOPMENT OF IMMUNOCOMPETENT ANIMAL MODELS FOR SIMULTANEOUS AND SEPARATE TESTING OF DRUGS ON *ONCHOCERCA* AND *LOA LOA* MICROFILARIAE, AND *ONCHOCERCA* ADULT WORMS

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Onchocerciasis is the second leading infectious cause of blindness globally, with over 99% of all patients residing in tropical Africa. The only currently recommended drug for treatment of the disease, ivermectin, kills the microfilariae (mfs) of the causative parasite, *Onchocerca volvulus*, but also *Loa loa* mfs in the blood of co-infected patients, resulting in severe adverse events and fatalities in some cases. Ivermectin does not kill the adult worms, which can linger on for more than 14 years, aggravating the burden of the disease. The search for new drugs against onchocerciasis has been seriously hampered by the lack of suitable animal models. The present study was aimed at developing immunocompetent small animal models that can better mimic the natural infection and that can be used to simultaneously or separately test new drugs on *L. loa* and *Onchocerca* ochengi mfs and adult *O. ochengi* worms. The co-infection model would permit selection for drugs that kill only *Onchocerca* and avoid fatalities in *L. loa* co-endemic areas during mass drug administration with current microfilaricides. Of several animals tested, the Mongolian gerbil (*Meriones unguiculatus*) was the only one permissible to both parasite species, with good parasite recoveries and excellent viabilities recorded on day 30 post experimental infections. Syrian hamsters were highly permissible to *Onchocerca* mfs and adult worms, but not to the *L. loa* mfs. Treatment of the gerbil co-infection with ivermectin at 150 µg/kg body weight resulted in complete elimination of *L. loa* mfs in blood and *O. ochengi* mfs in skin, and viability reduction of *L. loa* mfs in the peritoneum. Further development of the models is on-going, but so far we have developed and validated a new small animal co-infection model for the development of onchocerciasis drugs that do not kill *L. loa* mfs, and established suitable small animal hosts for adult *Onchocerca* worms.

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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL 7-AMINO PYRAZOLOPYRIMIDINE COMPOUNDS POSSESSING POTENT ANTI-*WOLBACHIA* ACTIVITY FOR THE TREATMENT OF ONCHOCERCIASIS AND LYMPHATIC FILARIASIS

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Filarial nematodes are a group of human pathogens that affect more than 157 million people worldwide, contributing to serious public health and socio-economic challenges. These parasites are responsible for the Neglected Tropical Diseases lymphatic filariasis and onchocerciasis, which is the second leading infectious cause of blindness. The main causative agents of these diseases are the nematodes, *Wuchereria bancrofti and Onchocerca volvulus* respectively. These infections are currently treated using mass drug administration programmes with chemotherapeutics donated by large pharmaceutical companies. Elimination however, remains hampered by insufficient activity against adult worms and serious adverse effects. The nematodes responsible for causing filarial diseases, share an essential endosymbiotic relationship with the bacterium, *Wolbachia*. Although the exact nature of this relationship is not understood, Anti-*Wolbachia* therapy has been identified as a viable treatment for filarial diseases, which delivers safe macrofilaricidal activity with superior therapeutic outcomes compared to current anti-filarial drugs. Doxycycline is the current gold standard for Anti-W*olbachia* activity, but requires treatment for at least four weeks and is contraindicated in children under 9 years of age and pregnant women. The Anti-*Wolbachia* (AWOL) drug discovery and development programme aims to identify alternative drugs which are suitable for a wider patient range and shorter treatment plan. This work describes the development of Anti-W*olbachia* agents of the pryazolopyrimidine chemotype. Despite high potency demonstrated by the original hit, DMPK experiments highlighted poor metabolic stability. Organic synthesis has enabled functionalization of key positions within our template, generating a broad library of compounds of which many analogues possess nanomolar activity against *Wolbachia in vitro* as well as display improved DMPK parameters. Further work is in progress to achieve our goal of providing a candidate that can achieve a 90% *Wolbachia* reduction in fewer than 14 days of treatment in the *in vivo* model system.

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ECONOMIC COSTS AND BENEFITS OF SCALING UP DISABILITY PREVENTION FOR LYMPHATIC FILARIASIS ACROSS INDIA

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Lymphatic filariasis (LF) is endemic in 73 countries, with 68 million people infected, of whom 36 million suffer serious disability (17 million with lymphedema, 19 million with hydrocele). Repeated acute attacks of fever and disabling pain (acute dermatolymphangioadenitis or ADLA) aggravate lymphedema and prevent work for 4-7 days per attack. The Global Programme to Eliminate Lymphatic Filariasis (GPELF) has two goals: interrupting LF transmission by 2020, and caring for people already infected through morbidity management and disability prevention (MMDP). By 2014, 60 countries had ongoing mass drug administration to end LF transmission, but only 24 had begun MMDP, in part due to its perceived high cost and low return. Simple, low-cost interventions at the community level, including instruction in limb washing and provision of soap, topical antibiotics, and antifungals can reduce ADLA and slow progression of lymphedema. MMDP programs attenuate disability and productivity loss. For Khurda District, Odisha State, India, we estimated lifetime medical costs and earnings losses due to chronic lymphedema and acute dermatolymphangioadenitis (ADLA) with and without a community-based limb-care program. The program would reduce economic costs of lymphedema and ADLA over 60 years by 55%. Savings of US$ 1 648 for each affected person in the workforce are equivalent to 1 258 days of labor. Per-person savings are more than 130 times the per-person cost of the program. We then estimate the costs of scale-up for all Indian states for community-based programs of limb care for lymphedema. India has great diversity in levels of wages (and thus foregone earnings from disability that prevents working), prevalence of lymphatic filariasis, health systems, NGO involvement, and other factors that influence community health programs. In spite of the diversity of conditions, our cost estimates demonstrate the long-term economic benefits of simple limb care and provide an economic rationale in addition to the ethical mandate for MMDP, the second pillar of GPELF.

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APPLICATION OF ULTRASONOGRAPHY TO DETECT PERITONEAL FILARIAL DANCE SIGN IN PRECLINICAL RODENT *BRUGIA MALAYI* MACROFILARICIDAL DRUG SCREENING MODELS

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Ultrasonography (USG) has been successfully used in placebo-controlled clinical trials to evaluate macrofilaricidal (curative) drug efficacy in onchocerciasis and lymphatic filariasis. Here we describe the application of a portable ultrasound machine (SonoSite MTurbo) to detect 'filarial dance sign' (FDS) in preclinical *Brugia malayi* rodent drug screening models. In these models, defined numbers of *B. malayi* adults were implanted into the peritoneum or, alternatively, variable *B. malayi* adult burdens were established from a unit intraperitoneal inoculum of infectious stage larvae either within inbred Severe-Combined ImmunoDeficient mice or outbred *Meriones unguiculatus* (Mongolian) gerbils. USG successfully detected FDS of mixed sex or single sex adult worm burdens to a degree of sensitivity of a single female worm or 2 male worms. USG could also be applied to semi-quantify worm loads based on strength and multiplicity of FDS signal within different peritoneal anatomical locations. In both non-blinded and blinded preclinical drug studies, USG detection of peritoneal FDS has subsequently been utilised to accurately predict macrofilaricidal outcome. This technique could therefore be highly beneficial in refining and reducing the number of animals used during drug screens and accelerating preclinical macrofilaricidal drug by being able to more rapidly detect drug efficacy by longitudinal exam of the same study group without the necessity of invasive surrogate filarial viability sampling.

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INVESTIGATING EARLY INFECTION STATUS OF THE FILARIAL PARASITE *BRUGIA MALAYI* IN THE CAT, THE LABORATORY MODEL FOR LYMPHATIC FILARIASIS

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Human lymphatic filariasis (LF) is a mosquito-borne disease primarily caused by the parasitic nematodes *Wuchereria bancrofti* and *Brugia malayi*. These parasites are a major cause of morbidity globally, with an estimated 120 million people infected. *Brugia malayi* is the preferred laboratory model for LF due to *W. bancrofti* requiring the use of primate hosts. Currently, the domestic cat is utilized as the primary non-rodent animal model for *B. malayi*. However, on average only 25%-50% of felines become patent, so a method of early detection would be invaluable. Currently, the only test to determine infection status is the detection of circulating microfilariae, which are usually detectable 4-6 months post-infection. In other filarial parasites such as *Dirofilaria immitis*, the Enzyme Linked Immunosorbent Assay (ELISA) is used to detect circulating female uterine antigen. Recently, it was suggested that heat treatment of serum or plasma may dissociate the antibody-antigen complex, potentially releasing the antigen so that it may be detected. Due to the close relationship of these filarial worms, there could be detectable cross-reactivity after heat treatment for *B. malayi* antigen in these capture-antibody tests. We hypothesized that we would be able to detect circulating antigen after heat treatment in the serum of these infected cats. Ten male domestic cats were infected by subcutaneous injection of 400 *B. malayi* third-stage larvae. Serum was collected at key time points post-infection. Both heat-treated and room temperature serum was tested for circulating antigen using the DiroCHEK® ELISA kit. Of the six cats that became microfilaremic, five tested antigen-positive, whereas only one cat with a low microfilaremia tested antigen-negative. These data may indicate a methodology other than microfilarial counts may be used to detect *B. malayi* infections in cats. Furthermore, heat treatment of serum could expose epitopes that cross-react with the antibody used in commercial *D. immitis* tests.

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CELLSCOPE-*LOA*: DISTRICT-WIDE DEPLOYMENT OF A POINT OF CARE TOOL FOR THE PREVENTION OF POST IVERMECTIN SERIOUS ADVERSE EVENTS IN *LOA LOA* ENDEMIC AREAS

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Because of the marked adverse effects (functional impairment) and serious adverse events (SAEs, occasionally fatal but more often with potentially irreversible neurologic manifestations) that can occur when

Loa loa microfilariae (mf) levels exceed 8,000 mf/mL and 30,000 mf/mL, respectively, implementation of ivermectin (IVM)-based elimination programs for lymphatic filariasis (LF) and onchocerciasis in areas where loiasis is co-endemic has been extremely problematic. Identifying those individuals "at risk" for such SAEs would allow them to be excluded from IVM community treatment and prevent SAEs. This strategy, termed "Test and not Treat" (TNT), relies on the development of a rapid field-friendly test to quantify *L. loa* mf in peripheral blood. To this end, we developed a mobile phone-based video microscope (CellScope-Loa) that automatically quantifies *L. loa* mf in whole blood in less than 2 minutes without the need for conventional sample preparation or staining. Between August and October 2015, a field evaluation was conducted in a health district of Central Cameroon to assess the performance of the Cellscope-Loa in comparison to examination of a calibrated blood smear (the current standard method to assess *L. loa* mf densities). Among the 15,298 participants, 226 (1.5%) had mf densities above 30,000 mf/mL, when assessed by calibrated thick smear. There was a strong correlation (rho=0.84, p<0.0001) between mf densities estimated by the CellScope-Loa and those measured by the calibrated thick smear. Receiver operating characteristic (ROC) analysis demonstrated that the CellScope-Loa could identify individuals harboring > 30,000 mf/mL with 94.0 and 99.6% sensitivity for CellScope-Loa thresholds set at 20,000 and 10,000 mf/mL, respectively. Most importantly, it had a negative predictive value (probability that the mf density is actually below 30,000 mf/mL) of 99.9 and 100% for the same threshold values. The TNT strategy based on the Cellscope-Loa is an extremely promising and practical approach to the safe implementation of large-scale treatment for LF and onchocerciasis in *L. loa* co-endemic areas.

1116

SYNERGY OF ALBENDAZOLE AND RIFAMPICIN COMBINATION THERAPY IN A MURINE INFECTION MODEL OF HUMAN LYMPHATIC FILARIASIS

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An estimated 120 million people are infected by lymphatic filariasis throughout the tropics leading to a profound public health and socio-economic burden in severely affected communities. Wolbachia is an essential endosymbiont of the filarial nematodes *Wuchereria bancrofti*, *Brugia malayi* the causative agents of lymphatic filariasis. Doxycycline is currently the gold standard for the targeting of Wolbachia in lymphatic filariasis chemotherapy. However, the current drug regimen is a 100-200 mg/day doxycycline dose given for 4 to 6 weeks to patients. The A·WOL consortium plan to reduce the current treatment time to 7 days or less to improve drug regimen adherence and to reduce drug resistance and costs of treatment. To achieve a rapid 7-day or less kill rate of Wolbachia, a number of drug combinations will be employed. These include different tetracyclines (Doxycycline and minocycline) rifamycins (Rifampicin or Rifapentine), Moxifloxacin as well as anti-helminthic drugs. The complexity of multiple drug combinations necessitates a rational approach in the identification and choice of the best treatments in in-vivo models and translating the animal treatments in the lab into clinical trials on the field. In this current study we apply a rational drug development approach using our on in our murine infection model of *B. malayi* and pharmacokinetic (PK) analysis to investigate the synergy of Albendazole and Rifampicin combination therapy on the macrofilaridal and anti-Wolbachia efficacy. Pharmacokinetic modelling and simulation allowed the administration of rifampicin dosages equivalent to a standard 10 mg/Kg or 600 mg dose or a 35 mg/Kg super-dose and albendazole equivalent to a 400-800mg clinical dose in our murine infection model of *B. malayi*, making drug exposure and efficacy results clinically relevant in comparison to traditional efficacy studies. We have found synergistic interaction between rifampicin and albendazole for both macrofilaricidal and anti-Wolbachia activities and have used PK analysis and parasitological methods to dissect the origins of these interactions.

1117

FACTORS PREDICTING TRANSMISSION ASSESSMENT SURVEY OUTCOMES FOR LYMPHATIC FILARIASIS

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National programs are progressing towards elimination of lymphatic filariasis (LF) as a public health problem. Nearly 300 transmission assessment surveys (TAS), population-based cluster surveys to determine whether prevalence has been lowered to a level at which mass drug administration (MDA) can be stopped, supported by USAID have been implemented in 14 countries. Since both failing TAS and continuing to implement MDA have financial and opportunity costs, TAS should be conducted at an appropriate time. A key question, which has not yet been analyzed using survey data, is therefore which factors increase the likelihood of passing TAS. We performed logistic regression analysis to examine whether the odds of passing TAS was related to baseline prevalence, number of MDA rounds implemented, or median epidemiological coverage. The analysis included data from 14 countries implementing 296 stop-MDA TAS between 2012-2015. Of these TAS, 90% of districts passed. We found that passing TAS was significantly associated with both baseline prevalence (OR 0.945, CI 0.915-0.976) and median epidemiological coverage (OR 1.044, CI 1.008-1.082) at alpha =0.05. While the number of MDA rounds was not significantly associated with passing TAS, it was important to control for as otherwise it confounded the relationship between baseline prevalence, median coverage, and passing TAS. The R-square value was low (0.0714), however; this indicates that this model does not include all of the factors that affect the likelihood of passing TAS. Ongoing analysis will incorporate additional factors that may affect the likelihood of passing TAS, such as vector species, diagnostic tests used to determine eligibility for TAS, and consecutive versus missed rounds of MDA, among others. These results confirm that it is important to achieve high coverage when implementing MDA, especially in districts with high baseline prevalence, and additional rounds of MDA may be necessary. National programs can increase the likelihood of passing TAS—and therefore achieving elimination—by implementing high-quality MDA throughout the program, rather than only in response to a failed TAS.

1118

THREE-DIMENSIONAL VISUALIZATION OF THE INTERNAL ARRANGEMENT OF ONCHOCERCAL (*ONCHOCERCA VOLVULUS*) NODULES USING HIGH-RESOLUTION MAGNETIC RESONANCE IMAGING

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Adult stages of *Onchocerca volvulus* live in subcutaneous or deep nodules. For descriptive biology or drug effect assessment purpose, the nodules are generally processed using either histology (fixation and section, followed by staining) or enzymatic digestion (incubation in collagenase to eliminate host tissue and isolate adult worms). Non-invasive detection of adult *O. volvulus* using ultrasound has also been used, but has little indications

because of the low optical resolution of the nodule content. None of these techniques enable to have a tridimensional view of how an onchocercal nodule is organized, and how the different worms arrange themselves relative to each other. Here, we had the opportunity to examine nodules using high-resolution magnetic resonance imaging (MRI). The nodules had been placed in a fixative just after their collection, and stored in the latter for about 20 years before the present study. To reduce the background noise and artifacts during image acquisition, nodules were immersed in Fluorinert FC-77 liquid, which is a proton-free fluid with low water solubility and similar magnetic susceptibility to the tissue. MRI experiments were done using a 9.4 Teslas apparatus equipped with a MAGNEX TS1276D, a Quadrature Volume Coils 400 MHz RF43 and associated with a VnmrJ Imaging acquisition system. 3D gradient echo images were acquired during14 hours with 100 ms repetition time, 4.44 ms echo time, 8 averages, a 30° flip angle, a 40 x 20 x 20 mm3 field-of-view and a 512 x 256 x 256 matrix. 3D reconstruction was processed using Myrian 1.21.1 (Intrasense, Montpellier, France) on the basis of DICOM data, in both Maximum Intensity Projection and average rendering modes. This study is a proof of concept that MRI can provide clear images of adult worms in onchocercal nodules fixed for many years. These results warrant further developments including adapted MRI coil and fine image analysis to assess the worm's viability. Studies could be conducted with recently collected nodules that have not been stored in a fixative, as well with small animals (rodents) naturally or experimentally infected with various filarial species.

1119

IDENTIFICATION OF NEW MACROFILARICIDAL COMPOUNDS FOR TREATMENT OF ONCHOCERCIASIS

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Current efforts to control and eliminate onchocerciasis are hindered by the lack of compounds that target the adult worm stage. In a joint collaboration with DNDi, academia and Celgene, a pipeline was established to identify macrofilaricidal compounds. To date, more than 400 compounds have been screened *in vitro* against *Onchocerca gutturosa* adults, identifying 120 compounds with EC_{50} <1μM 40 of which having EC_{50} <100nM. From this set of 400 compounds, a select set of 160 compounds were tested against both *O. lienalis* microfilariae and *Onchocerca gutturosa* adults, identifying 43 compounds with specific activity against the adult parasites *in vitro*. Active compounds with EC_{50} in the 0.015-1μM range and suitable pharmacological profiles were prioritized for *in vivo* testing. 23 lead candidates were tested by oral gavage in mice that harbored adult worms of the rodent filarial nematode *Litomosoides sigmodontis*. Two compounds significantly reduced the *L. sigmodontis* adult worm burden by 98 and 93% after 10 days of TID treatment and 1 day of BID treatment, respectively. Presence of microfilariae in the treated animals suggest that both compounds do not have a strong microfilaricidal effect. Current efforts to further assess the impact of both compounds on microfilariae in the *L. sigmodontis* jird model are scheduled. The current study demonstrates the successful establishment of a screening cascade which resulted in the identification of two promising novel macrofilaricidal compounds. The identification of such macrofilaricidal compounds which lack microfilaricidal effects are ideal candidates for the treatment of onchocerciasis, as they have a reduced risk for microfilariae-driven adverse events.

1120

COMPARISON OF THE ONCHOCERCIASIS OV16 IGG4 RAPID TEST AND OV16 ELISA AMONG CHILDREN IN TOGO: EXPERIENCES WITH A NEW SURVEILLANCE TOOL

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The Alere SD BIOLINE Onchocerciasis IgG4 rapid test (RDT) is a new field tool for on-site identification of antibodies to the Ov16 protein of *Onchocerca volvulus,* the parasite that causes river blindness. WHO recommends using Ov16 ELISA to decide when to stop mass treatment with ivermectin. In 2015, in preparation for a move towards onchocerciasis elimination, the Ministry of Health of Togo used the Ov16 RDT in a national survey to obtain preliminary data on the prevalence of antibodies to Ov16 in school-age children and to compare Ov16 RDT to Ov16 ELISA. The survey was integrated with an NTD impact assessment. At each of 1126 schools serving as NTD sentinel sites, a convenience sample of 8 children age 6 to 9 years had finger-stick blood drawn for Ov16 RDT. A subset of children provided blood spots on filter paper (DBS) for testing by Ov16 ELISA. In total, 9007 children were tested by RDT, of whom 60 (0.7%) were positive. DBS were obtained from 2600 children. Ov16 ELISA testing is ongoing; of 294 RDT-negative samples tested to date, 50 of 294 (17%) were positive by ELISA. The significant discrepancy between RDT and ELISA results prompted additional investigations. Confirmatory Ov16 ELISA testing will be conducted at a US laboratory. The protein glutathione S-transferase (GST) is fused to the Ov16 protein. To assess whether Ov16 ELISA positives may be due to antibody cross-reactivity with GST, a GST-specific ELISA will be run on a subset of samples. To assess whether the RDT was properly conducted in the field, it will be repeated using the same DBS samples as for ELISA, using a modified protocol for testing DBS on Ov16 RDT. Application of the expectation maximization algorithm to our ELISA findings may improve classification of results. The 60 children who tested positive by RDT and a subset of those who are RDT-/ELISA+ will be revisited to document residency and travel history, repeat the RDT, and conduct skin snip testing with treatment if indicated. Resolution of these test discrepancies is important for onchocerciasis elimination in Togo. These findings highlight some of the challenges of employing these tests and our results should illuminate where pitfalls lie.

1121

DESIGN AND EVALUATION OF A HEALTH EDUCATIONAL BOARD GAME FOR THE CONTROL OF SOIL-TRANSMITTED HELMINTHIASIS AMONG PRIMARY SCHOOL CHILDREN IN ABEOKUTA, NIGERIA

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Despite repeated annual treatment with anti-helminthic drugs, soil-transmitted helminthiasis (STH) remains an important factor in school children morbidity in sub-Saharan Africa as school children are rapidly re-infected within 3 months after treatment. We designed a health education board game "Worms and Ladders" inscribed with health education and STH preventive messages and evaluated its potential for promoting good hygiene practices among school children for the integrated control of STH during mass drug administration (MDA). The evaluation employed a randomized control trial across six primary schools in Abeokuta, Nigeria. A total of 372 pupils were enrolled in the study, of which 212 were in the intervention group in three schools, and 160 were in the control group in three schools. Baseline knowledge, attitude and practices (KAP) relating to STH were obtained with a questionnaire followed by the collection

of fresh stool samples for STH diagnosis. All the participants were then treated with Albendazole according to their height. The designed "Worms and Ladders" board game were introduced and distributed to the intervention group to play for six months under the supervision of their class teachers. No game was given to the control group to play. Prevalence of STH dropped from 25.0% to 10.4% in the intervention group and 49.4% to 33.3% in the control group at three months' post treatment. The prevalence further dropped to 5.6% in the intervention group but increased to 37.2% in the control group at six months' post treatment. There was a significant difference ($p<0.05$) in post-treatment prevalence among the two groups. Knowledge, attitude and practices on transmission, control and prevention of STH significantly improved ($p<0.05$) from 5.2% to 97.9% in the intervention group compared to (6.2% to 7.1%) in the control group. The "Worms and Ladders" board game have shown its potential to promote good hygiene behaviour among school children and should be integrated into Mass deworming programme to prevent reinfection.

1122

RISK FACTORS ASSOCIATED WITH PREDISPOSITION TO SOIL-TRANSMITTED HELMINTH INFECTION IN SOUTHERN MYANMAR

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Southeast Asia has a substantial burden of soil-transmitted helminths (STH). In Myanmar, a mass drug administration (MDA) programme targeting STH in school-aged children (SAC), in conjunction with a community-wide MDA programme targeting lymphatic filariasis, has been implemented for the last 3-5 years and coverage has been consistently high. Past STH studies have identified the phenomenon of predisposition to STH infection in human hosts; individuals predisposed to STH infection may act as persistent reservoirs of infection. Parasitological data were analysed in conjunction with epidemiological data, collected during two MDA rounds in two villages in southern Myanmar (n=584). Baseline STH prevalence was 27.05%; 5.14% for *Ascaris lumbricoides*, 16.95% for *Trichuris trichiura* and 8.9% for hookworm. There was no statistical difference in presence of predisposition to STH infection (defined here as positive STH infection in both rounds) between the two villages. Preliminary analysis suggests that predisposition to STH infection is associated with individual adults' main form of employment ($P<0.0001$), if the main toilet is shared with other households ($P=0.003$) and household income ($P=0.03$). There was no evidence to suggest that predisposition was associated with age or gender. Aggregation of predisposed individuals was observed at a household level. It is concluded that predisposition to STH infection is associated with socioeconomic and water and sanitation hygiene (WASH) factors. Further analysis will include identifying factors associated with predisposition to single STH species infections.

1123

IMPLEMENTATION AND EVALUATION OF A QUALITY AND SAFETY TOOL FOR AMBULATORY STRONGYLOIDIASIS PATIENTS AT HIGH RISK OF ADVERSE OUTCOME

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Although simple intestinal strongyloidiasis is curable, infections are frequently asymptomatic and unknown, leading to potential adverse patient outcomes upon immunosuppression. Given the potential complexity of care of patients with strongyloidiasis, a safety tool may help to improve patient outcomes and standardize care for strongyloidiasis patients. We developed a novel safety tool, and implemented it in June 2015. Our aim was to evaluate the utility of the tool using a retrospective chart review. Patients diagnosed with strongyloidiasis were identified through our clinic "Special Access Programme" log from January 1, 2013 to December 31, 2015. Patients were categorized as treated for strongyloidiasis pre-implementation of the tool (prior to June 2015), or post-implementation of the tool (June 2015 to December 2015). Outcome measures included loss to follow-up; documentation of seroreversion post-treatment; documentation of stool clearance post-treatment, if positive at baseline; and screening for factors known to confer risk of hyperinfection and dissemination (e.g, HTLV1 infection) During the study period, 37 patients were treated for strongyloidiasis: 23 males (62%), and 14 females (38%). Median age was 44 yrs (range 5-85 yrs). 24 patients were treated pre-implementation of the tool, while 13 were treated after implementation. Loss to follow-up after treatment occurred in 25% of patients pre-implementation, and for no patients in the assessible post-implementation group ($p=0.148$). Proof of seroreversion occurred in 47% of assessible patients pre- implementation, and 66% of assessible patients in the post implementation group ($p=0.635$). Proof of stool clearance and seroreversion occurred in 50% (2/4) of patients pre-implementation, and 50% (1/2 assessible patients) post implementation ($p=1.000$). Evaluation of HTLV-1 co-infection occurred in 33% of patients pre-implementation, and in 69% post-implementation ($p=0.021$). Our findings suggest that a safety tool may of benefit in preventing future adverse outcomes due to strongyloidiasis, though a larger prospective evaluation is warranted.

1124

STRONGYLOIDIASIS IN ONTARIO: INFORMING THE DIAGNOSTIC EVALUATION BY ESTABLISHING THE NUMBER NEEDED TO EXAMINE (NNE)

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In order to provide a diagnostic strategy that offers sensitivity, specificity, as well as larval staging and quantification, clinical guidelines suggest using a combination of serologic and microscopic diagnostic tests for confirmation of strongyloidiasis. We aimed to evaluate the performance of stool microscopy, serology, and real time PCR (qPCR) for the optimal diagnosis of strongyloidiasis at our reference laboratory. We included all specimens submitted for O&P examination and *Strongyloides* serology between April 1, 2014 and May 31, 2015. All stool specimens positive for any stage of *Strongyloides stercoralis*, as well as 5-times that number of random stool specimens negative for *Strongyloides* were included in our verification of a *Strongyloides*-specific qPCR assay. Positivity rates were calculated, and the total number of examined specimens divided by the number of positive specimens was calculated as the "Number Needed to Examine" (NNE). During the enrolment period, 17,933 stool specimens were processed for O&P examination, 14 of which were positive for *Strongyloides* larvae, yielding an overall NNE for stool microscopy of 1281. During the enrolment period, 3258 specimens were processed for *Strongyloides* serology, 200 of which were reactive (6.1%), 210 indeterminate (6.5%), and 2848 non-reactive (87.4%), yielding an NNE for reactive serology of 16. Two patients shedding larvae and known to the laboratory as undergoing iatrogenic immunosuppression had negative serology. qPCR was positive in 10 of 12 (83.3%) stool specimens containing larvae, and negative in all stool specimens without larvae by microscopy. There was no cross-reactivity of *Strongyloides*-specific qPCR to other common stool protozoa or helminths. In the absence of immunosuppression, larval burden in strongyloidiasis is low, limiting the utility of microscopy for diagnosis, and favoring serologic testing. However, false negative

serology can occur in those with hyperinfection necessitating a combined diagnostic approach. The qPCR assay was insufficiently sensitive to replace microscopy for detection of larvae.

1125

EOSINOPHILIA, ANEMIA AND INTESTINAL PARASITES IN CHILDREN FROM RURAL COMMUNITIES OF VENEZUELA

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A cross-sectional epidemiological survey was conducted in four rural communities in the north of Venezuela. One stool sample of school-age and unschooled children between 0 and 15 years old was requested (n=438/909) and examined for ova and parasites, using direct smear (428/438) and Kato-Katz techniques (n=400/438). Hemoglobin (Hb), hematocrit and relative eosinophil value (REV) were also determined (n=803/909). A questionnaire was performed before processing the samples. Eosinophilia (REV ≥ 5%) was detected in 90,6% (n=763/842) and was more prevalent in UBP (94,5%) and Palo Negro schools (95,1%) p <0,001. Anemia (Hb < 11,5 g/dl) was demonstrated in 7% (n= 56/803) with the highest prevalence in TK (21,7%) and EH schools (30,4%) p <0,001. *Giardia intestinalis* (n=86/428; 20,1%), *Ascaris lumbricoides* (n=26/400; 6,5%) and *Trichuris trichiura* (n=19/400; 4,8%) infections did not show a significant statistical association with anemia (Fisher's exact p=0.284; p=0.532; p=0.167 respectively). Mean parasitic loads for both helminths were low according to WHO cut offs. We reccomend that in settings of developing countries, with high prevalence of eosinophilia and low prevalence of intestinal nematodes according to stool examination, the burden of soil-transmitted helminthiasis may also take in consideration other variables suchs as unsatisfied basic needs and undiagnosed strongyloidiasis, to assess the most adequate therapeutic intervention in order to reduce the associated morbidity.

1126

PREVALENCE OF STH AT A COASTAL AREA IN INDIA - ROLE OF STUDENTS' HYGIENE PRACTICES, SCHOOL AND HOME SANITATION FACILITIES

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Soil transmitted helminth infestation is a global health concern affecting two billion population worldwide. Mainly three species viz. *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm inflict considerable morbidity and mortality amongst infected persons. Present study was conducted among school children of Allepey city, Kerala state, India after having prior consent from respective authority. Sample size was estimated according to WHO guidelines as 200-250 children per ecological zone. Children were informed about the purpose of study. Information regarding their social and personal hygiene practices was recorded with the help of semi structured questionnaire and they were provided with a container to bring stool sample on the following day. Samples collected were subjected to examination for helminthic eggs using Kato-Katz technique. Statistical analysis was done by using Chi-square test. Total 219 samples were collected in which 79 (36%) were positive. *A. lumbricoides* infection was found in all samples. 76 (95%) samples had monotypic infection. data was analyzed to find out the factors associated with occurrence of STH infection. The factors like not having household latrine, not washing hands before meal and practice of eating food fallen on ground/unwashed vegetables/fruits were found associated with having STH infection among children and this was found statistically significant. Significant association was found between presence of anaemia and having STH infection. Preventive chemotherapy along with social awareness about good hygienic practice go hand in hand in reducing morbidity associated with STH. Hand washing and in-house sanitary latrine qualify as the most important preventive measures.

1127

HIGH PREVALENCE RATES OF SOIL-TRANSMITTED HELMINTHS IN CHILDREN WHO RECEIVE MASSIVE DRUG ADMINISTRATION IN A REMOTE AMAZONIAN COMMUNITY IN PERU

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Following the recommendations from the WHO to control soil-transmitted helminth (STH) infections, a school-based deworming program with albendazole 400mg oral single dose every 3 months was recently implemented in the Amazon rainforest of Peru. In order to evaluate the prevalence of STHs in children from a community within the program's scope, we collected stool samples from 2-14 years old children from *Padre Cocha*, Loreto. All samples were analyzed for the presence of *Ascaria lumbricoides, Trichuris trichiura,* hookworm *and Strongyloides. stercoralis*; by using the following techniques: spontaneous sedimentation in tube, Kato-Katz, modified Baermann and agar plate culture. Stool samples of their mothers were also collected and examined. Additional information including weight, height, sociodemographic and epidemiological risk factors (i.e. water supply, sanitation and hygiene practices) were collected by interview. Out of 124 children, 32 (25.8%) were infected with one or more STH. Twenty (16.1%) had *A. lumbricoides;* 2 (1.6%), hookworm; 2 (1.6%), *T. trichiura;* and 13 (10.5%) had *S. stercoralis.* Malnutrition was present in 72 (60.8%) of the children, although no association was found with STH infection (OR:1.02; IC:0.41-2.63). Among risk factors, walking barefoot was associated with any STH (OR:3.47; IC:1.16-12.5). Prevalence of STHs was higher in the mothers from the children infected (36.4%) compared with the mothers from those uninfected (14.1%) (p<0.02). In conclusion, children from this particular Amazonian community have high prevalence rates of STHs even after an intensive MDA program. The mothers infected with STHs could play an important role in the dissemination of the eggs and larvae (*S. stercoralis*) from these parasites, which could eventually contribute to reinfections. High prevalence of *S. stercoralis* prompt further evaluation of the inclusion of ivermectin within the local MDA program. Integration of more control measures for STHs -including clean water supply, adequate sanitation services and hygiene education- should be taken into account to develop a successful program, particularly in remote populations from this region in Peru.

1128

AN AUTOCHTHONOUS CASE OF GNATHOSTOMIASIS ACQUIRED IN QUEENSLAND, AUSTRALIA

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Gnathostomasis is an uncommon foodborne zoonosis, which is caused by infection with the larvae of *Gnathostoma* spp, most commonly *Gnathostoma spinigerum*. In Australia, *G. spinigerum* has been identified in cats and bandicoots, and *G. hispidum* in wild pigs. Only two locally acquired gnathostomiasis cases have been reported from Australia. We present the case of a previously well 30-year-old man presenting to a Darwin Hospital with left forearm swelling and eosinophilia, positive

gnathostomiasis immunoblot and an epidemiological plausible link to infection from undercooked mudcrabs caught in Yeppoon, Queensland after heavy flooding.

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MODELING COGNITIVE DEFICITS IN SOIL-TRANSMITTED HELMINTHIC INFECTION USING GOLDEN SYRIAN HAMSTER

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Soil-transmitted helminthic (STH) infection, which includes *Ascaris*, whipworm, and hookworm (*Ancylostoma duodenale*, *Necator americanus, and A. ceylanicum*), remains a significant public health issue today. Previous studies in humans, both retrospective and prospective, have linked STH infections with anemia, nutritional deficiency, growth stunting, and impaired memory and cognition, potentially leading to lower socioeconomic status and perpetuation of poverty. However, the results to date have been conflicting with regard to the nature of cognitive deficit and benefit of anthelmintic therapy. Using Gold Syrian hamsters, which are susceptible to acute *A. ceylanicum* infection, we wanted to isolate, observe, and study potential cognitive defects from confounding factors normally present in work with humans. Hookworm infection did not impair the hamster's ability to recognize previously encountered objects (Novel Object Recognition task), but did impair performance on a spatial memory task (Displaced Object Recognition task; control vs infected, p=0.02). Further, the severity of deficit was dependent on infection burden (control vs severe infection, p=0.01) and the impairment appeared to be reversible with clearance of infection. Accordingly, acute hookworm infection resulted in deficits in cognitive function, which resolved with the clearance of STH infection. It will be important to determine if the cumulative effects of repeated infections produce long-term cognitive deficits.

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SOIL TRANSMITTED HELMINTHS IN BENIN: EVIDENCE OF COUNTRYWIDE HOOKWORM PREDOMINANCE

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From 2013 to 2015, mapping of soil-transmitted helminths (STH) was conducted in all 77 of Benin's communes as part of the baseline surveys for schistosomiasis and STH. The aim of the mapping was to provide epidemiological data needed to develop a national strategy for the control of neglected tropical diseases (NTDs) in Benin by 2020. In each commune, 5 schools were purposively selected; and in each school 50 children (25 girls and 25 boys) ages 8 to 14 were randomly selected. In total, 19,250 stool (from 9,625 girls and 9,625 boys) from 385 schools were examined using Kato-Katz technique. STH are present in all districts. National prevalence of STH is 22.74% (95% CI 2.00% - 62.80%) and 58.44% of the communes (45/77) have prevalence of ≥20% and therefore require mass drug administration (MDA) for STH. Four species of STH (Hookworm, *Ascaris, Trichuris* and *Enterobius*) were observed with intra-specific and inter-specific variation in the prevalence and density of the parasites. Hookworm constitute the highest proportion of STH parasites and the prevalence was 17.40% (95% CI 0.40% - 60.00%; n=76); *Ascaris* 5.35% (95% CI 0.40% - 26.40%; n=62); *Trichuris* 1.15% (95% CI 0.40% - 9.60%; n=37); and *Enterobius*: 1.92% (95% CI 0.40% - 18.80%; n=32). Hookworm was present in 100% of the surveyed communes with a national mean prevalence of 17.14% (95% CI 0.40% - 60.00%). Most infections were of light density except in the communes located in the department of Atacora. This mapping provides a global view of the epidemiological pattern of STH needed for the implementation of a control strategy (which could potentially include MDA and the provision of potable water, sanitation and health education). Multiple infections with several STH are commonplace among the school-age children surveyed, possibly due to poor hygiene and sanitation. In Benin, the high prevalence of hookworm and its predominance in all communes calls for a strengthening of maternal and child health policy through health education and routine screening of pregnant women. In addition, STH MDA should be tailored to target not only school-age children, per the standard guidance, but also women of child-bearing age.

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REPEATED ROUNDS OF MASS DEWORMING ADMINISTRATION STILL LEAVE HOUSEHOLD CLUSTERING OF SOIL-TRANSMITTED HELMINTH INFECTIONS

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Kenya is one of the many countries endemic for soil-transmitted helminth (STH) infections. Since 2012, a national school-based deworming programme has provided preventive anthelminthic treatment to over 6 million children annually. While this control strategy reduces overall infection levels, infection bounces back soon after treatment administration. In order to improve the efficiency of control programmes, further knowledge on the factors driving reinfection is still required. A study conducted in 2014 in western Kenya investigated the clustering of STH infections at the village and household levels. A cross-sectional survey was conducted in four rural villages near Bungoma Town, targeting over 1000 residents from 2-81 years of age. Epidemiological data were collected at two time-points, at study baseline and three months post-treatment with 400mg albendazole. Treatment was administered to all study participants to simulate a mass drug administration campaign. At study baseline, *Ascaris lumbricoides* and *Necator americanus* infections had an overall prevalence of 9.8% and 6.7% (n=763), respectively, and *A. lumbricoides* was significantly more prevalent in one of the villages (17.7% vs. 6.0%, 4.0% and 3.9%; Pearson X^2=35.7, p<0.001). Three months post-treatment, levels of *A. lumbricoides* and *N. americanus* infection were reduced to 1.8% and 1.4%, respectively, with no significant difference between villages. Clustering at household level was observed for *A. lumbricoides*, with 1.3 and 1.75 persons infected per house at the first and second time-point, respectively. This clustering was found to be associated with predisposition to *A. lumbricoides* infection, with 62.5% of the infected households at the second time-point also having participants infected at the first time-point. This study highlights the importance of household clustering and predisposition to infection in the maintenance of *A. lumbricoides* infection in endemic communities, a factor to take into account if elimination of STH infections is to be successful.

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UNDERSTANDING THE EPIDEMIOLOGY OF HOOKWORM INFECTION IN A LOW-TRANSMISSION SETTING IN SOUTHERN INDIA: ANALYSIS OF DATA FROM A CLUSTER-RANDOMIZED MASS DEWORMING TRIAL

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Hookworms are a leading cause of malnutrition and anemia in resource poor settings. Periodic mass deworming of at-risk populations (particularly school-going children) with anthelminthic drugs remains the cornerstone of hookworm control efforts worldwide. Reinfection rates following treatment are high, suggestive of an untreated reservoir of transmission. Also, recent modeling-based estimates suggest that school-based deworming may have limited impact in interrupting the community transmission of hookworm infections. In a recently concluded cluster-randomized community-intervention trial of a modified population-based mass deworming strategy, 8681 participants aged 2-70 years, residing in 45 tribal villages in southern India were randomized into 3 groups: one received one round of treatment with albendazole (400 mg) at month 1, the second received two rounds of treatment at months 1 and 2, and the third received four rounds of treatment - two rounds at months 1 and 2, followed by another two at months 8 and 9. Stool samples collected from a subset of participants pre- and post-intervention were tested for hookworm by microscopy to evaluate the effect of the treatment with albendazole and at 3-monthly intervals for one year to test for re-infection. The baseline prevalence of hookworm infection ranged from 2-44% (overall prevalence: 19%; 95% CI: 16-21%); majority of infections (90%) were of low intensity (<2000 epg). The prevalence and intensity of infection increased with increasing age. Following deworming, hookworm prevalence decreased after the first two doses, but remained stable thereafter. Data from this trial will be used to estimate the key parameters for hookworm transmission models. Deterministic, age-structured models will be fitted to assess the transmission dynamics of hookworm infection. The effect of individual, household and village-level predisposition to hookworm infection will be explored. The models derived from this study will help identify the drivers of hookworm transmission in a low-transmission setting and can be used to further refine the end-game strategy for hookworm control.

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MODELLING THE EFFECT OF PATTERNS OF ADHERENCE AND NON-ADHERENCE TO TREATMENT IN PURSUIT OF HELMINTH ELIMINATION BY MASS DRUG ADMINISTRATION

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In mass drug administration (MDA) aimed at elimination of disease and/or disease, sufficient treatment coverage is essential for success. Non-adherence to treatment is a barrier to achieving adequate coverage. A critical question concerns the likelihood of non-adherence at one round of treatment predisposing to non-treatment at subsequent rounds. In addition the transmission dynamics may be affected by patterns of treatment or non-treatment over multiple rounds - for example, individuals untreated over multiple rounds of MDA may act as a reservoir of infection. We address the importance of these issues through the construction and analysis of a stochastic computational model in which the effects of systematic non-adherence on coverage and treatment pattern are examined in detail and conclusions drawn about the importance of non-compliance to achieve policy goals.

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TO WHAT EXTENT IS PREVENTIVE CHEMOTHERAPY FOR SOIL-TRANSMITTED HELMINTHIASIS 'PRO-POOR'? EVIDENCE FROM THE 2013 DEMOGRAPHIC AND HEALTH SURVEY, NIGERIA

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Health equity is a guiding principle for the control and elimination of neglected tropical diseases (NTDs), including those addressed through so-called 'preventive chemotherapy' (PC), such as soil-transmitted helminthiasis (STH). NTD advocacy messages frequently emphasize the 'pro-poor' potential of PC, with the aim of 'rescuing the bottom billion.' Available evidence suggests that poverty, lack of education, and residence in rural areas are all significant risk factors for STH. Little information is available, however, on the extent to which PC reaches those at highest risk within STH-endemic communities. To identify demographic and socioeconomic factors associated with drug coverage for STH, we analyzed data from the 2013 demographic and health survey from Nigeria. In this nationally-representative survey, mothers were asked whether their young children received deworming medication during the previous 6 months. We conducted multivariable logistic regression that controlled for child age, gender, immunization status and having received vitamin A supplementation in the previous 6 months. Overall, 19.9% of children 6-59 months of age were reported to have received deworming medication in the previous 6 months. Reported six-month deworming prevalence was 15.2% in rural areas, compared to 28.4% in urban areas. Prevalence of deworming increased linearly with level of maternal education (9.4% for children whose mothers had no formal education, compared to 42.5% with more than secondary education) and wealth quintile (7.9% for the lowest wealth quintile, compared to 39.1% for the highest). Deworming prevalence remained significantly associated with maternal education (odds ratio [OR] 2.2, 95% confidence interval [CI] 1.8-2.7), wealth (OR 2.3, CI 1.9-2.7), and urban residence (OR 1.6, CI 1.3-2.0) in the multivariate analysis. These results highlight systemic challenges to actualizing a 'pro-poor' NTD policy and raise questions about the degree to which current NTD policies and practices, particularly for STH, actually reach those at greatest risk.

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THE SECOND GLOBAL NGO DEWORMING INVENTORY: ASSESSING SOIL-TRANSMITTED HELMINTHIASES TREATMENT REPORTING

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Affecting nearly one billion children globally, soil-transmitted helminthiases (STH) is the most common parasitic infection. To combat the disease, the World Health Organization (WHO) target for 2020 is to reach ≥75% of children at-risk of STH with regular preventive chemotherapy (PC). Ministries of Health (MoH) provide routine STH treatment reports to the WHO PCT Databank to monitor treatment coverage. In 2014, 47% of at-risk children were reported to receive PC. Non-governmental organizations (NGOs) also play a key role in administering PC. However, according to the first Global NGO Deworming Inventory (DI) for data of 2009 and 2010, over a third of the NGO administered treatments were unreported to WHO in 2010. To assess progress made in improving coordination of NGO-MoH-WHO reporting of STH treatments, a second DI was conducted after a 5

year period. From August to October, 2015, 40 NGOs were surveyed, of which 17 (43%) reported administered STH treatments in 2014. NGO-delivered treatments were again compared with those reported by MoHs to WHO. Comparing 2014 to 2010, the total number of reported STH treatments increased from 261 million to 441 million globally; treatments delivered by NGOs increased from 65.4 million (25%) to 187.2 million (42%); and the number of NGO treatments unreported to WHO decreased from 23.3 million (36%) to 10.5 million (6%). The NGO unreported treatments constitute 2.4% of the global total in 2014, compared to 8.9% in 2010. These findings demonstrate improved NGO-MoH data reporting and collaboration at the country level. Only six countries accounted for >90% of NGO unreported STH treatments in 2014. While the DI is not intended to provide an alternative data collection process, periodically comparing the DI and PCT Databank can further strengthen reporting by prioritizing support to countries with large gaps in reporting. Combining data from these two sources highlights important reporting gaps, demonstrates the effect of systematized efforts to reduce them, and helps to increase confidence in the accuracy of national treatment coverage reporting to the global level of children at-risk of STH infection.

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GUIDANCE IN DESIGNING SURVEYS FOR MONITORING SURVEYS FOR MONITORING THE PROGRESS OF SCHOOL-BASED DEWORMING PROGRAMS TO CONTROL SOIL-TRANSMITTED HELMINTHIASIS

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There is a worldwide upscale in school-based deworming programmes to control the morbidity caused by soil-transmitted helminths (STH). However, there is a lack of guidance in designing surveys to verify whether these programmes progress as anticipated. We expanded an existing 2-level hierarchical model that accounts for variation in egg counts within individuals due to the egg counting procedure (level 1; Poisson distribution) and between individuals within a school due to host-parasite interactions (level 2; negative binomial distribution (NB)), to a 3-level model that also accounts for clustering of STH infections between schools (level 3; NB or zero-inflated NB distribution). In addition, we adapted the model for variation in the effectiveness of deworming programmes at both the individual and the school level. To maximize the flexibility in survey design, the framework was worked out for the examination of both individual and pooled stool samples. From the derived formulae to estimate the variance, we updated the methodology to calculate the number of schools and the number of individuals per school required for assessing (i) the intensity of infections, (ii) the effectiveness of programmes and (iii) the absence of infections for any scenario of disease epidemiology, diagnostic strategy and programmatic effectiveness. To bridge the gap between this mathematical framework and the end-users we further developed an online interface that guides the user in designing an appropriate survey without the need of prior mathematical or statistical knowledge. At the meeting we will briefly outline the underlying mathematical framework. Subsequently, we illustrated its applications using available data on the effectiveness of deworming and the related costs to diagnosis STH infections in epidemiological surveys in Eastern Africa. Finally, we will demonstrate selective features of the online tool.

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IMPACT OF HELMINTH INFECTIONS DURING PREGNANCY ON HUMORAL VACCINE IMMUNOGENICITY IN INFANTS

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Infection with helminths is considered as a neglected tropical disease and is a major public health problem especially in the tropics. The influence on cognitive and physical development as well as on the immune system is well recognized. Recent studies showed that individuals infected with helminths have a reduced antibody response to vaccination. Furthermore, there is evidence that infants born to helminth-infected mothers display changes of their immune system that might lead to a reduced antibody response towards vaccines. In this ongoing study we investigate the influence of maternal helminth infection on the immunogenicity of vaccines administered within the national Expanded Program on Immunization (EPI) in Gabon. Infants of mothers either with or without helminths are compared (NCT02714348; BMBF 01KA1307). More than 300 mothers have been enrolled and helminth status assessed prior to delivery. Blood has been collected at delivery from the mother and the cord, and additionally from the infants at 9 months and 12 months of age. The antibody profile to the vaccines are measured with ELISA. Here we present preliminary study data.

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INTESTINAL PARASITIC INFECTIONS IN HIV INFECTED AND NON-INFECTED PATIENTS IN A HIGH HIV PREVALENCE REGION, ADAMAOUA-CAMEROON

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The magnitude of intestinal parasitic infection in acquired immunodeficiency syndrome patients requires careful consideration in the developing world where poor nutrition is associated with poor hygiene and many tropical diseases. Studies have addressed this issue in Cameroon, mainly in the low HIV prevalence settings. This study aimed to determine the prevalence of intestinal parasites in HIV/AIDS patients in Adamaoua (with HIV prevalence 5.1%) Stool and blood specimens from HIV/AIDS patients and control group were screened respectively for intestinal parasites and for HIV antibodies. Intestinal parasites were identified using direct microscopy, formalin-ether concentration, Ziehl Neelsen and giemsa stains methods. Out of 235 participants recruited among patients consulting at hospital, 69 (29.24%) were HIV positive, Thirty-one of them treatment naïve (44.93%). The prevalence of intestinal parasites was 32.34%. Out of 69 HIV/AIDS patients, 31.88% (22/69) were infected with intestinal parasites, while 32.53% (54/166) of the HIV negative patients were infected with intestinal parasites. The parasites detected in the population included: *Blastocystis hominis* (18.30%), *Entamoeba histolytica* (6.36 %), *Entamoeba coli* (5.96 %), *Endolimax nanus* (3.83%), *Iodamoeba buetschlii* (2.13%), *Crystosporidium* spp (2.98%), *Trichomonas intestinalis* (1.70%), *Embadomonas intestinalis* (0.43%), *Cyclospora cayetanensis* (0.43%), *Ascaris lumbricoides* (0.43%). There was no difference between the prevalence of intestinal parasites

amount people living with HIV and people living without HIV. The parasitic density instead was higher in the group of HIV positive patients compared to HIV seronegative people. 38 out 69 HIV infected patients were on ARV and 31 were still treatment naïve. Being on treatment or not did not affect infection by intestinal parasites. Poor hygiene conditions were associated to parasites infections (p=0). In conclusion, HIV patients should be screened routinely for intestinal parasites and treated for their overall well being. Emphasis should be placed on their hygiene as well.

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OLDER AND FORGOTTEN; SEXUAL BEHAVIOR AND PERCEIVED HEALTH STATUS AMONG HIV POSITIVE AND NEGATIVE MENOPAUSAL WOMEN IN NIGERIA

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Not only are people living longer with the Human Immunodeficiency Virus, but there is also a significant increase in older individuals becoming infected. Women in the menopausal transition may constitute a group that is vulnerable to HIV infection with less likelihood of using a condom and low perception of the risk of HIV infection with inadequate knowledge of HIV transmission and prevention. This study assessed sexual behavior and perceived health status among HIV positive and negative menopausal women in Ibadan, Nigeria. Focus group discussions were conducted among HIV positive and negative menopausal women attending the ARV and General Outpatient clinics at the University College Hospital Ibadan, Nigeria with the use of a focus group discussion guide. Opinions of discussants on condom use, having multiple sexual partners, knowledge of HIV transmission and prevention and perceived health status were explored. Ten focus group discussions were conducted among women aged 40 and 60 years in each of the two groups. Data was analysed thematically. A total of 90 HIV positive and 92 HIV negative women aged between 40 to 60 years were sampled. While all the women opined that condom use protects against STIs and unwanted pregnancy, condom use was low among both groups. More HIV negative women opined that having multiple sexual partners was unbecoming and any older woman with multiple sexual partners is promiscuous, however, HIV positive older women strongly affirmed that men often run away when they disclose their HIV status to them, hence the need for multiple sexual partners. Knowledge of HIV transmission and prevention was poor among both groups. Interestingly, more HIV positive women perceived their health status as good, compared to HIV negative women. Coping strategies include belonging to a support group and seeking information from health care workers. Menopausal women are vulnerable to HIV infection and its impact on this group should not be ignored. There's a need for risk reduction strategies and health promoting interventions that will help these women in coping with the double burden of HIV infection and ageing.

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PREVALENCE OF PREECLAMPSIA AMONG HIV-POSITIVE PREGNANT WOMEN AS COMPARED TO HIV-NEGATIVE WOMEN IN IBADAN

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Preeclampsia is a common complication of pregnancy and a major cause of maternal morbidity. Pathogenetic explanations for preeclampsia include; maladaptation of the immune system to paternal antigens and exaggerated maternal inflammatory response to trophoblastic tissue. Immune deficiency, induced by HIV or any other cause, could therefore inhibit a tendency to immune hyper-reactivity and thus theoretically prevent the development of preeclampsia. The study aimed to explore the role of the immune theory of pre-eclampsia by comparing the prevalence of preeclampsia among HIV-positive and HIV-negative pregnant women. The study is a cross-sectional survey of pregnant women, beyond 28 weeks gestation, who delivered at the University College Hospital, Ibadan, Nigeria between 1st October 2011 and 31st December 2011. Data was collected using a pre-specified proforma. Analysis was done using SPSS version 17.0 and p-value was set at <0.05. A total of 766 women who gave birth during the period of the study met the inclusion criteria. Among the cohort, HIV prevalence rate was 7.2% while preeclampsia was 10.7%. None of the HIV-positive women had preeclampsia. This study concluded that the prevalence and perhaps, risk of developing preeclampsia is reduced among HIV positive women. This is similar to other studies done in various countries in the world.

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HTLV AND HIV CO-INFECTION AMONG KEY POPULATIONS, DOMINICAN REPUBLIC

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HTLV and HIV coinfections are not well characterized among most at-risk populations. Overall, there has been a decline in HTLV research in the past 24 years with few studies reporting current data on its prevalence in endemic countries. Past studies have shown that HTLV-1 and HIV coinfection causes increased HTLV-1 seropositivity and subsequent risk for tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM) and other neurological diseases in addition to reduced survival time. Based on the fact that HTLV and HIV share the same modes of transmission, the purpose of this investigation was to estimate the seroprevalence of HTLV IgG and HIV antibodies and to establish the prevalence of coinfection among two key populations, transactional sex workers (SWs) and drug users. A demographic, stratified sample of 200 sera was randomly selected in four high burden regions of Santo Domingo, Dominican Republic. Informed consent was obtained from each participant and each received pre- and post-counselling about HIV and HTLV transmission. Blood samples were drawn from each participant and screened for HIV and HTLV-1/2 IgG antibodies using ImmunoComb®II (Orgenics, Israel) products. Overall weighted seroprevalence of HTLV-1/2 IgG antibodies was 13.91% (CI: ±6.32%) in men and 10.59% (CI: ±6.54%) in women and for HIV-1 was 13.91% (CI: ±6.32%) in men and 17.65% (CI: ±8.10%). Of those HTLV positive, 50% of those men and 44.44% of those women were coinfected with HIV and half of whom were SWs. Seroprevalence of both HTLV and HIV antibodies detected among heterosexual SWs (33.33%) appears to be the most important route of transmission. Results call attention for more public health preventive strategies among key populations in the Dominican Republic and further investigation on the neurological complications experienced and clinically relevant effect of HTLV-1 on HIV positive patients in endemic regions.

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FACTORS ASSOCIATED WITH DEFAULTING FROM CARE AMONG ADULTS ON ANTI-RETROVIRAL TREATMENT PROGRAM IN ONDO STATE, NIGERIA, 2015

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Nigeria has one of the highest burden of Human Immunodeficiency Virus (HIV), with a prevalence of 3.4%, and over 700,000 on antiretroviral therapy (ART). Defaulting from care is an emerging threat to successful control of HIV. This study explored factors associated with default among adults on ART in Nigeria. An unmatched 1:2 case control study was conducted at Federal Medical Centre, Owo, Ondo State. Cases were

adults who defaulted clinic visit at least three consecutive times and have not returned to care, while controls were consistent in clinic visit for at least 6 months. Defaulters identified from the clinic register were interviewed at home. Controls were selected from the ART clinic using systematic sampling technique. Semi-structured questionnaire was used to collect data on respondents' socio demographics, disclosure status and knowledge on ART treatment. Four in-depth interviews (IDI) with defaulters were conducted to document the barriers to retention in care. A total of 102 cases and 204 controls were enrolled. Respondents mean age was 41.4±10.3 years and 118(38.6%) were males. Defaulting from treatment was associated with non-disclosure of status to partner (AOR: 2.8; CI 95%: 1.6-4.9), receiving fewer counselling sessions (AOR: 2.3; CI 95%: 1.3-4.2), perception that quality of service received was poor (AOR: 2.6; CI 95%: 1.4-4.7), and sub-optimal quality of life (AOR: 2.7; CI 95%: 1.5- 4.8). Major reasons for defaulting included: traveling out of base 10(67%) and lack of support from workplace 9(50%). IDI revealed clinic not operating on weekend as a cause of defaulting. In conclusion, the clinic management team increased the number of patient counselling sessions and reviewed its content to include training on disclosure techniques. Commencing weekend clinic days was recommended.

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MOLECULAR AND CLINICAL IMMUNE STATUS OF HIV EXPOSED BUT UNINFECTED (HIV EU) INFANTS COMPARED TO CONTROL HIV UNEXPOSED (HIV UU) INFANTS: A COHORT STUDY IN KISUMU DISTRICT, KENYA

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Kenya has one of the world's greatest HIV burdens; nearly 1.5 million adults are infected. Due to increased access to antiretroviral therapy and mother to child transmission interventions implemented in Kenya, the number of babies infected with HIV is low. But as the number of HIV positive adults in Kenya remains steady and transmission to infants is decreased, there is now a growing population: HIV exposed but uninfected (HEU) infants. These infants, while healthier than HIV positive infants, have increased morbidity and mortality compared to HIV unexposed uninfected (HUU) infants. We hypothesized that *in utero* exposure to HIV and/or the chronically activated maternal immune environment resulting from HIV infection affects the fetal immune system leading to prolonged elevation of pro-inflammatory biomarkers, decreased antibody production, and increased clinical events of infants. This study is investigating the following aims: (1) Determining the magnitude and duration of elevated pro-inflammatory biomarkers in HEU vs. HUU infants using MagPix analysis of plasma samples at birth, 6, 10, 14, 18 weeks, and 6, 9, and 12 months (2) Determining the prevalence and magnitude of antibodies against multiple *Plasmodium falciparum* antigens by serology up to two years of age and (3) Characterizing the clinical events (such as pneumonia, meningitis, malaria) experienced by HEU vs. HUU infants over the first two years of life. Preliminary results suggest that there is no significant difference in the levels of pro-inflammatory biomarkers present in plasma of HEU vs. HUU infants at all of the time points tested between birth and one year. These results suggest that perhaps the immune system of HEU infants is actually more stable than initially hypothesized. This finding is further supported by preliminary results from the clinical database that suggest there is no significant difference in the number of sick visits, severe infections, or hospital admissions between the HEU and HUU infants.

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HIV EPIDEMIOLOGY AND COVERAGE OF HIV HEALTH SERVICES IN GEM COUNTY, SIAYA COUNTY, WESTERN KENYA, 2013-2014

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According to the Kenya AIDS Indicator Survey (KAIS) of 2012, the former Nyanza province of Kenya had the highest HIV prevalence among persons aged 15-64 years; 15.1% (against the country's national average of 5.6%) with 18.3% among females and 13.9% among males. In the same survey, 71.3% and 79.9% of persons had ever been tested for HIV in the past country-wide and in Nyanza province respectively. Among all males, 91.2% were circumcised country-wide and 66.3% in Nyanza. HIV prevalence in uncircumcised and circumcised men was 16.9% and 3.1% and 25.9% and 8.1% in Kenya and Nyanza respectively. Among the HIV infected, 89.3% were enrolled in HIV care countrywide of whom 88.6% were on Cotrimoxazole Preventative Therapy (CPT); CPT coverage was 90.0% in Nyanza. We present more recent results of a home based HIV counseling (HBCT) survey in Gem Sub-County, Siaya County (one of the five counties in the former Nyanza province). We reviewed data for persons aged between 15 and 64 years of age who participated in the HBCT Survey, between June 2013 and August 2014. We compared HIV prevalence and coverage of selected HIV health services for former Nyanza province in KAIS 2012 to HBCT. Of 21,879 persons who participated in the survey; 63% were female. Among all participants, 19,417 (89%) had ever been tested for HIV in the past of whom 2084 (10.7%) tested positive; majority (98%) of HIV positive persons had been enrolled into HIV care and were on CPT (98%). Among the larger group (89.3%) who had tested negative for HIV in the past, HIV prevalence during survey testing was 4.1 % (n=704) and 7.1 % (n=171) among those who had never been tested. The overall HIV prevalence was 13.8 %. HIV prevalence was significantly higher among females compared to males (16.4% vs. 9.4%). Among all men, 64.5% had been circumcised. HIV prevalence was significantly higher among circumcised compared to uncircumcised men (4.9% vs. 11.8%). The current HIV prevalence in this region reflects the country's downward trend in HIV prevalence and maybe due to increased coverage of HIV health services. More comprehensive comparisons should routinely be made to illustrate trends in the HIV response.

1145

USING THE LIVERPOOL HIV ICHART TO PREDICT THE SPECTRUM OF DRUG-DRUG INTERACTIONS IN A COHORT OF HAART- EXPOSED PERSONS LIVING WITH HIV (PLHIV) IN A TREATMENT CENTRE IN SOUTH-SOUTH NIGERIA

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Drug-drug interaction (DDI) occurs where two or more drugs interacts in a manner that alters their therapeutic effects. HAART may interact with co-medications in HIV patients and present severe complications. Objective We used the Liverpool iCHART application to describe the pattern of drug-drug interactions between HAART and other medications in a cohort of HAART-exposed PLHIV at the University of Port Harcourt Teaching Hospital. This was a review of client records at the HAART clinic of the University of Port Harcourt Teaching Hospital in the South-South region of Nigeria conducted in September 2014. Folders of 480 patients who reported for clinic consultations and drug refills were selected via systematic sampling over a 20 day period. We used simple random sampling to identify two drug prescriptions from client folders. A data extraction form was used to record data on socio-demographic details, co-morbidities, co-prescribed drugs as well as the specific HAART regimen employed. The Liverpool HIV iChart Application was used to screen for possible interactions such

as potential drug interactions and clinical significant drug interactions (CSDI) between the ARV drugs and co-medications. Results The study involved record from 157(33.1%) male and 321 (66.9%) female PLHIVs with mean age of 38.82±9.68 years with ages ranging from 20 to 84 years. Prevalence of potential drug interactions (PDI) was as high as 463 (96.5%) while the prevalence of CDSI was 216 (45%). All client prescriptions reviewed using the app had elements in the green indicating no interactions, 464 clients (96.7%) had elements in amber indicating presence of potential interactions for which caution was needed while 48 clients (10%) of elements of their drug treatments in the red/danger zone indicating an absolute contra-indication to co-administration of HAART and the particular co-medication. In conclusion, the Liverpool HIV ICHART is an inexpensive and useful resource for predicting drug-drug interactions for PLHIV on HAART. Its routine use is advocated in HIV treatment centres especially in resource poor settings.

1146

DIAGNOSIS OF TOXOPLASMOSIS REACTIVATION IN HIV PATIENTS IN URINE USING NANOPARTICLE TECHNOLOGY

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The most devastating opportunistic diseases associated with HIV are those that affect the central nervous system (CNS). Their nonspecific presentation makes diagnosis difficult even in the best-resourced settings. In many cases, diagnosis by exclusion is the only option. *Toxoplasma gondii* is one such malady. Thus we present a point of care western blot for the detection of *T. gondii* antigen, SAG1. *T. gondii* seroprevalence varies by continent and socioeconomic status, but is estimated to be highest in Latin America ranging from 39-90%. An estimated 33% of patients with advanced immunosuppression and previous seropositivity for *T. gondii* will develop toxoplasmic encephalitis (TE). Diagnosis of *T. gondii* in HIV positive patients is difficult without CT or MRI in resource-poor settings. PCR of cerebral spinal fluid (CSF) has a sensitivity of 12-to-70%. Safe collection of CSF is difficult in locations without medical facilities and expertise. Obtaining a culture of the parasite is possible, but requires at least 6 weeks for completion, rendering results diagnostically irrelevant. When TE is treated early, the disease boasts a 90% clinical response rate. Thus, a rapid point of care test with high sensitivity and specificity, using an easily obtained body fluid is absolutely necessary if LMIC treatment is to surmount the aforementioned diagnostic challenges. Nanoparticle-concentrated urinary-antigen diagnostic assays are noninvasive, safe, and inexpensive; they provide a rapid, accurate, and precise parasitological test in resource-constrained zones where PCR is not readily available. Sensitive and specific urinary tests combined with clinical assessment will aid in the diagnosis and treatment of TE in HIV-infected patients. Briefly, nanoparticles were circulated in murine or human urine samples, collected, and the contents eluted. Said contents were separated by polyacrylamide electrophoresis and detected by western blot. Results illustrate detection of all positive and negative samples in accordance with previous qPCR results. We are intrigued by these results and will continue pilot testing this assay as patient samples become available.

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COULD ACCELERATION TOWARDS GLOBAL 90:90:90 HIV TARGET ALONE END TB BURDEN AMONG UNDIAGNOSED PEOPLE LIVING WITH HIV? A FOUR YEAR PRE- AND POST-ISONIAZID PREVENTIVE THERAPY IMPLEMENTATION COMPARATIVE DATA FROM COMPREHENSIVE HOSPITAL IN NORTHWESTERN NIGERIA

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Early access to HIV testing and counselling provides opportunity to start antiretroviral therapy (ART) and Isoniazid Preventive Therapy (IPT) for eligible people living with HIV (PLHIV). There is paucity of data that demonstrate comparative programmatic analysis of the impact of IPT intervention on the incidence of TB among PLHIV on ART and those not on ART. In August 2013, the PRO-ACT project funded by USAID and implemented by Management Science for Health started implementation of 6 month of IPT in General Hospital Koko, northwestern Nigeria. The objective of the study is to assess and compare impact of IPT in reducing TB incidence among PLHIV on ART and those previously undiagnosed and not on ART. Using the Hospital TB register between July 2011 to September 2015, TB/HIV co-infection cases were extracted into 2 groups i.e. group 1/pre-IPT (July 2011-July 2013) and group 2/post-IPT implementation era (September 2013-September 2015). TB/HIV co-infected cases in each group were then categorized into 2 cohorts each. Cohort 1 are those not on ART, not on IPT, not previously diagnosed as HIV positive until they develop TB. Cohort 2 patients are those previously diagnosed HIV positive, not on IPT but on ART before they develop TB. Cohort 3 was on IPT, not previously diagnosed as HIV positive until they develop TB and not on ART. Cohort 4 was on IPT, previously diagnosed as HIV positive before they develop TB and on ART. Data from the 4 cohorts were then compared to demonstrate impact of IPT and ART in reduction of TB burden and incidence among PLHIV. The lowest incidence (23.4%) of new TB cases was found in post-IPT era among cohort 4 while the worst incidences of new TB cases of 53.3% and 76.6% were found among cohorts 2 and 3 respectively. The study demonstrates the combined multiplier effects of both IPT and ART in reducing the incidence of TB among PLHIV and increased TB incidence among previously undiagnosed HIV cases in Northwestern Nigeria. In line with global 90:90:90 target, scaling up HIV testing and counselling will ensure early diagnosis as entry point for improved access to lifesaving ART and IPT thereby reducing the incidence of TB among undiagnosed HIV cases.

1148

THE GENETIC VARIATION WITHIN SUB-SAHARAN POPULATIONS ENDEMIC TO HUMAN AFRICAN TRYPANOSOMIASIS

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Human African trypanosomiasis (HAT) is one of the neglected tropical diseases that affect thousands of individuals in sub Saharan Africa. Within these populations, some individuals are highly susceptible to infection whereas others remain asymptomatic. The TrypanoGEN consortium was setup to study the Human genetic determinants of disease susceptibility

and trypano-tolerance in 6 African countries. In order to identify loci that are associated with disease, whole genome sequencing was carried out on 250 individuals from five populations; Guinea (GUI), Ivory Coast (CIV), Cameroon (CAM), Democratic republic of Congo (DRC) and Uganda (UGA) to discover SNPs for a planned Genome wide association study (GWAS). Approximately 2 million SNPs within the TrypanoGEN population samples were called. Principal component analysis (PCA) and Admixture analysis were used to identify population structure that could confound the GWAS. The samples clustered into 4 distinct groups; West African Bantu (CIV & GUI), Central African Bantu (DRC), Ugandan Bantu (UGB) and Ugandan Nilotics (UGN). Further population structure analysis showed that the Uganda Bantu (UGB) had substantial admixture with the Nilotic (UGN) ancestry (approximately 50%), were as the Nilotic are a distinct population with no Bantu admixture. There is some evidence that HAT is a relatively recent infection associated with agriculture and livestock domestication. Consequently, loci such as APOL1 associated with disease response may show signs of positive selection. Preliminary Fst analysis has already identified loci with high Fst which have known associations with malaria or methotrexate toxicity. An analysis of loci under positive selection that may be associated with response to trypanosomiasis will be presented.

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EVIDENCE OF AUTOCHTHONOUS CHAGAS DISEASE TRANSMISSION IN SOUTH TEXAS

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Chagas disease (*Trypanosoma cruzi* infection) is one of the most significant neglected tropical diseases affecting the Americas. There are an estimated 8-9 million prevalent cases worldwide with an estimated 300,000 infected people residing in the United States. Chagas disease is often referred to as the 'silent killer' due to its long (up to 3 decades) asymptomatic period. During this period, progressive cardiac damage can occur in up to one-third of infected individuals. In fact, sudden cardiac death is the first presenting symptom in 35% of those with cardiac manifestations. Due to concern for transmission of the parasite from infected blood products, national blood donation screening became mandatory in the United States in 2007. Since screening started, 47 seropositive donors have been identified in the greater San Antonio area. From this convenience sampling, we aimed to gain a better understanding of the transmission sources for Chagas positive donors in south Texas. Our case investigation found 71% (12 out of 17) of enrolled donors had evidence of acquiring the infection locally. Risk factors for disease in those with autochthonous transmission included rural residence, outdoor occupations, and outdoor hobbies. Of most concern, 58% (5 out of 12) had evidence of Chagasic cardiac disease as detected on electrocardiogram. This study adds to the growing body of evidence for autochthonous Chagas disease transmission in the southern United States. In fact, from this population, we identified the largest percentage of autochthonous cases to date. This has important implications for public health officials and clinicians as our understanding of the global epidemiology of this disease changes. Following this presentation, the audience will have a better understanding of the epidemiology of Chagas disease in the southern United States.

1150

PAN-AMERICAN MIGRATION PROMOTES THE SPREAD OF PATHOGENIC *TRYPANOSOMA CRUZI* HYBRID STRAINS

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The principal reproduction strategy of *Trypanosoma cruzi*, the aetiological agent of Chagas disease, is the subject of an intense, decades-old debate. Despite the existence of two recent natural hybrid lineages (TcV and TcVI), which are sympatric with severe disease in southern endemic areas, a pervasive view is that recombination has been 'restrained' at an evolutionary scale and is of little epidemiological relevance to contemporary parasite populations. With improved sampling strategies, the geographical distribution of TcV and TcVI appears to be expanding. High resolution nuclear and mitochondrial genotyping of potential hybrid isolates from domestic vectors and human infections in Colombia was undertaken, in comparison to representative strains from across South America, to resolve their putative status as novel recombinants. All suspected Colombian hybrids were highly heterozygous, minimally diverse and possessed intact parental alleles at each loci. Compared to local Colombian isolates, hybrids were distinct from, but more closely related to, those identified in southern reference TcVI strains. Based on independent inheritance patterns of microsatellite loci, our data support the hypothesis that two recombination events led to the formation of TcV and TcVI. However, more private alleles among Colombian hybrids and the sharing of mitochondrial haplotypes between southern TcV isolates and a Colombian TcVI strain, suggests the evolution of these recombinant lineages may be more complicated than previously assumed. The origin of these Colombian hybrids is unclear; they are unlikely to be predecessors of southern TcVI strains, but were also not clear descendants, and may instead represent a sibling group, which diverged and anthroponotically dispersed northwards, following a single hybridization event between heterozygous southern TcII and TcIII isolates. We discuss the important implications the geographical range expansion of TcVI has for emergent human Chagas disease in Colombia, considering the successful, epidemic establishment of this low-diversity genotype among domestic transmission cycles in the South.

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EVIDENCE AND IMPORTANCE OF GENETIC EXCHANGE AMONG FIELD POPULATIONS OF *TRYPANOSOMA CRUZI*

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Many eukaryotic pathogenic microorganisms that were previously assumed to propagate clonally have retained cryptic sexual cycles. The principal reproductive mode of *Trypanosoma cruzi*, the aetiological agent of Chagas disease, remains a controversial topic. Strong linkage disequilibrium, deviations from Hardy-Weinberg allele frequencies and structuring of parasite populations into stable, distinct genetic clades have been used to argue that recombination has been restrained at an evolutionary scale and has little influence on contemporary field populations. However, with improved sampling strategies and the development of higher resolution nuclear and mitochondrial genotyping techniques, mounting evidence now indicate that natural hybridization in *T. cruzi* may be frequent, non-obligatory and idiosyncratic; potentially involving independent exchange of kinetoplast and nuclear genetic material as well as canonical meiotic mechanisms. Asymmetric mitochondrial introgression is emerging as a common feature of some transmission cycles, which given their crucial role in growth, development and metabolism, satisfies the elevated necessity to escape from Muller's ratchet, and may be exploitable as a method of host range extension. A clear understanding of the implications of genetic exchange for the ecological and geographical distributions and

pathological characteristics of *T. cruzi* strains is crucial to establish the epidemiological risk associated with hybrid genotypes, with respect to virulence, transmissibility and drug susceptibility. We discuss the growing number of field studies which now challenge the traditional paradigm of preponderate clonal evolution in *T. cruzi*, the caveats underlying why detecting genetic exchange is inherently complicated, given that parasites most likely to be recombining may be closely related and potentially indistinguishable, and describe experimental strategies which must be adopted to resolve these fundamental biological phenomena in trypanosomatids as we enter the post-genomic era.

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CHAGAS DISEASE IN PREGNANT WOMEN AND SCREENING BY PCR IN NEWBORNS FROM GUANAJUATO, MÉXICO

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Chagas disease is caused by an infection with the protozoan hemoflagellate *Trypanosoma cruzi*, and it is a major endemic health problem in Latin America. The congenital route is one of the main non-vectorial pathways of transmission, which can arise either in the chronic or acute phase of maternal infection. Serological screening of *T. cruzi* infection was performed in 520 pregnant women and newborns at the Hospital General Regional de León, Guanajuato, Mexico, between 2014 and 2015. Anti-*T. cruzi* antibodies were detected in 20 mothers (4%) by ELISA and HIA with four PCR-positive newborn cases. Risk factors were identified according to an epidemiological survey, and the most significant ($P < 0.050$) factors associated with *T. cruzi* infection were the building materials of dwellings, the presence of pets and dwellings located in rural areas. This study constitutes the first systematic study on congenital Chagas disease and the epidemiological risk factors in Guanajuato. Our results represent the probability of an incidence of 770 cases per 100,000 births during a period of 12 months, with a vertical transmission rate by 0.8%, which highlights the necessity to establish reliable serological and PCR tests in pregnant women to prevent vertical transmission. However, it is also important to follow-up the newborns from seropositive mothers for one year, which is necessary, as many children yielded negative results.

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TRYPANOSOMA CRUZI PREVALENCE AND SEROPREVALANCE IN A COHORT OF U.S. SERVICE MEMBERS IN SOUTH TEXAS

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Although the vast majority of the estimated 300 thousand U.S. residents infected with *Trypanosoma cruzi*, the causative parasitic agent of Chagas disease, are among immigrants from endemic areas of Latin America, autochthonous transmission is possible in some parts of the United States. A study is being conducted at Joint Base San Antonio-Lackland, Texas, to determine the prevalence and seroprevalence of Chagas disease among military members who may be at increased risk due to field training activities in an area where a large number of *T. cruzi*-infected triatomines have been identified. We offered voluntary enrollment to U.S. Air Force technical training students graduating from security forces training, Basic Military Training field instructors, and military working dog instructors. Volunteers completed a questionnaire regarding risk factors for Chagas disease and had their blood drawn. Real-time polymerase chain reaction (PCR) and two serologic tests (enzyme-linked immunosorbent assay [ELISA] for IgG and IgM and immunofluorescence assay [IFA] for IgG) were conducted on the collected samples. Descriptive statistics were used to analyze the prevalence and seroprevalence of *T. cruzi* and risk factors. A total of 473 individuals have been enrolled to date. Of the tests conducted (PCR [N=460]; ELISA [N=465]; IFA [N=465]), all have been negative for *T. cruzi*. The participants reported a mean of 7 weeks spent in a triatomine-endemic field environment, 273 weeks living or traveling in the Southwest United States and Latin America, and 15 weeks camping or hunting in the Southwest United States and Latin America. Chagas disease does not constitute a significant operational risk to service members who conduct or undergo field training in south Texas. The lack of positive cases may demonstrate the effectiveness of countermeasures that prevent triatomine bites (e.g., trainees are issued insect repellent and permethrin-treated bed nets) and/or the poor efficacy of stercorarian transmission of *T. cruzi*.

1154

TRANSMISSION DYNAMICS OF VISCERAL LEISHMANIASIS IN THE INDIAN SUBCONTINENT - A SYSTEMATIC LITERATURE REVIEW OF THE ROLE OF ASYMPTOMATIC LEISHMANIAL INFECTION, POST-KALA-AZAR DERMAL LEISHMANIASIS AND RELAPSE RATES

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As Bangladesh, India and Nepal progress towards visceral leishmaniasis (VL) elimination, it is important to understand the role of asymptomatic *Leishmania* infection (ALI), VL treatment relapse and post kala-azar dermal leishmaniasis (PKDL) in transmission. We systematically reviewed evidence on ALI, relapse and PKDL as potential reservoirs of infection. We searched multiple databases to include studies on burden, risk factors, biomarkers, natural history, and infectiveness of ALI, PKDL and relapse. After screening 292 papers, 98 were included. ALI, PKDL and relapse studies lacked a reference standard and appropriate biomarker. The prevalence of ALI was 4-17-fold that of symptomatic VL. The risk of ALI was higher in VL case contacts. Most infections remained asymptomatic or resolved spontaneously. The proportion of ALI that progressed to VL disease within a year was 1.5-23%, and was higher amongst those with high antibody titres and those who had a VL case in the family. The natural history of PKDL showed variability; 3.8-28.6% had no past history of VL treatment. About 49% of PKDL resolved spontaneously without treatment. The infectiveness of PKDL was 32-53%. Relapse following VL treatment occurred in 0.14-20% and the risk was higher with HIV co-infection. Modelling studies produced a range of scenarios. One model predicted that early diagnosis was unlikely to eliminate VL in the long term. Another model estimated the infectiveness of ALI to be 1-3% and that ALI contributed to 82% of the overall transmission, VL to 10% and PKDL to 8%. In contrast, another model predicted that VL cases were the main driver for transmission. Another model predicted that VL would be eliminated if the sandfly density was reduced by 67% by killing the sandfly or by 79% by reducing their breeding sites. Another model predicted VL elimination with 4-6y of optimal IRS or 10y of sub-optimal IRS and only in low endemic setting. There is a need for xenodiagnostic and longitudinal studies to understand the potential of ALI and PKDL as reservoirs of infection.

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MOLECULAR DETECTION OF *LEISHMANIA (VIANNIA) PANAMENSIS* IN ANTHROPOPHILIC AND ZOOPHILIC SANDFLIES FROM AN ENDEMIC FOCUS OF CUTANEOUS LEISHMANIASIS IN PANAMA

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Cutaneous leishmaniasis (CL) is a parasitic zoonosis prevalent in many rural areas of Panama. *Leishmania Viannia panamensis* is considered the main involved etiologic agent. The transmission of this parasitic disease is conditioned by the bite of infected sandflies, most of them belonging to the genus *Lutzomyia*. The abundance and diversity of these vectors in Panama is high with about 76 described species. However, only six of these species feed on human blood frequently (anthropophilic), and therefore have been considered important vectors. Little is known about the infection rates with confirmed *Leishmania* species in these putative vectors and even less in those considered zoophilic vectors, although the latter are abundant in many foci of transmission. In this study, sandflies were collected using light traps HP inside and around 24 houses from Trinidad de Las Minas, a rural community where CL transmission is high. More than 5,600 sandflies were collected. The most abundant anthropophilic especies were *Lu. panamensis* (967), *Lu. gomezi* (1,146) and *Lu. trapidoi* (1,151). Among the zoophilic vectors, *Lu. dysponeta* (490) and *Lu. triramula* (1,150) were the most frequently species found. Female sandflies were pooled in 5 to 10 individuals per species. *Leishmania* infection and species discrimination was performed by ITS-1 and kDNA PCR and by HSP70 PCR-RFLP and sequencing analysis respectively. The results confirm the high infection rate with *L. (V.) panamensis* in the anthropophilic vectors, predominantly *Lu. trapidoi*. Interestingly, it was also demonstrated the infection in the zoophilic *Lu. triramula* and *Lu. dysponeta* pools. This is the first molecular detection and identification of *L. (V.) panamensis* within naturally infected *Lu. triramula* and *Lu. dysponeta* from an endemic focus of CL in Panama. This finding concluded that *Lu. triramula* and *Lu. dysponeta* are susceptible to L. (V.) panamensis infection and suggest that more attention should be paid to zoophilic sandflies vectors regarding the ecoepidemiology of CL.

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EFFECT OF GEOGRAPHICAL DIFFERENCES, *TRYPANOSOMA CRUZI* INFECTION AND BLOOD MEAL ON MICROBIOME OF *TRIATOMA INFESTANS*

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The protozoan parasite *Trypansoma cruzi* causes Chagas disease, which is considered one of the neglected tropical diseases by the WHO. Chagas disease is primarily transmitted by triatomine bugs, which bites a human for a blood meal and defecates near the bite site. Infection occurs when the person rubs feces containing the parasite into the bite wound or a mucous membrane. We examined the fecal microbiome of *Triatoma infestans* bugs captured in Arequipa, Peru during vector control campaigns in 2011 and 2014-2015. Frozen, ethanol-preserved bugs were thawed, and fecal contents were expressed onto filter paper. DNA was extracted from the fecal spots using a modified phenol chloroform technique. Quantitative real time polymerase chain reaction (qPCR) was used to test for *T. cruzi* infection. Blood meal source for each bug was identified using conventional PCR targeting a 355-bp segment of the cytochrome B gene followed by Sanger sequencing. Sequences were run through the BLAST database on the NCBI website to identify the species of the bug's last blood meal. MEGA was used to align sequences and QIIME to examine genetic diversity and relatedness between samples. 16S ribosomal rRNA was amplified using universal primers and deep sequenced using Illumina technology. 90 insect fecal samples were extracted and sent for 16S sequencing and cytochrome B could be amplified from 58 of those. From the samples that amplified cytochrome B 74% (43/58) of those were found to have human mitochondrial DNA, 12% (7/58) had various rodent DNA, 9% (5/58) had dog DNA and 5% (3/58) had chicken DNA. We will compare diversity and composition of triatomine gut microbiome by *T. cruzi* infection status, bug stage, geographic location, and blood meal source. These findings may have implications for the development of new non-traditional vector control interventions.

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OUTCOMES OF A COMMUNITY INTERVENTIONAL-EVALUATIVE MODEL FOR NEGLECTED DISEASES IN EAST POKOT, KENYA

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Neglected tropical diseases (NTDs) are the primary barriers for low-income individuals to escape poverty. Population based cross-sectional surveys conducted in 2012 and 2013 in East Pokot, Kenya showed high seroprevalence of visceral leishmaniasis (VL) with 23 rK39-confirmed cases out of 1,324 screened, compounded by high rates of poverty, hunger, illiteracy and conflict. This necessitated the need to develop an interventional program using community strategies. Focusing on three administrative locations in the East Pokot sub-county, the ongoing model is comprised of public health education, provision of rapid diagnostics, screening and treatment, training of clinicians and community outreach workers, environmental control and operations research. Between 2015-2016, 552 rk39 rapid diagnostic kits (Bio-Rad) were distributed to seven health facilities. As a result, 326 individuals were screened for VL, of which 60 displayed rK39 positive results. A community-based screening of 441 individuals who meet clinical case-definitions identified 14 rk39-positive individuals. Seven coordination meetings were held with national, regional and local stakeholders. Ten health workers (laboratory technicians, nurses and clinicians) were trained on recognition, treatment, management and referral of potential VL cases. Fifteen community health volunteers were trained on recognition, referral of suspected VL cases and health education. Integrated public health education campaigns on VL and trachoma prevention and treatment were conducted in nine villages and six schools. Additionally, 200 VL educational posters were distributed to nearby schools, churches, health facilities and market areas. Integrated mobile clinics reached 516 patients. Vector control activities were conducted in five villages. An entomology assessment was conducted to investigate the distribution of the sandfly vector and the relationship between the ecology and socio-cultural context impacting disease. A mixed model for research and community-based interventions can be a successful approach in impacting NTDs in hard-to-reach populations.

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PREDICTORS OF CHAGAS INFECTION AMONG INDIGENOUS COMMUNITIES IN THE SIERRA NEVADA DE SANTA MARTA IN COLOMBIA

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Chronic Chagas cardiomyopathy is the main contributor to mortality in endemic regions and is caused by the protozoan parasite *Trypanosoma cruzi*. Many factors affect the susceptibility to the infection, including environmental, genetic, parasite-driven and vector-mediated. However, additional clinical features contribute to the determination of infections, and the aim of this study was to rank the importance of these factors for classifying participants as Chagas positive or negative in an indigenous community in Sierra Nevada de Santa Marta, Colombia. A cross-sectional case-control study was implemented, with 232 patients with positive dual Chagas serologies and 261 negative serologies enrolled. Among study participants residing in areas with greater than 10% infestation by *Triatoma dimidiata* (TD), 64% were positive for Chagas disease compared to 46% in areas with less than 10% infestation (chi-square p<.001). Variables associated with positive Chagas serologies were chest pain (37% vs. 18%, p<.001), paroxysmal nocturnal dyspnea (PND) (20% vs. 8%, p=0.02), syncope (24% vs. 9%, p<.001), edema (20% vs. 9%, p=0.05) and abnormal EKG (28% vs. 17%, p=.02). Participants with Chagas positivity were significantly older than participants that were negative for Chagas (33 years vs. 25 years, respectively, p<.001). Random forest classification was implemented by fitting 500 bootstrap aggregated trees with 5 variables randomly chosen among a list of 16 possible at each node. The top five variables according to the mean decrease in accuracy were infestation by TD, age, chest pain, syncope and PND. The top five variables with respect to mean decrease in Gini were age, infestation by TD, chest pain, syncope and edema. Abnormal EKG was in the top 10 for both importance measures. Older age, higher infestation of TD, chest pain, PND, edema, syncope and abnormal EKG are important indicators of potential Chagas infection in this region. These findings highlight the critical importance of vector-driven factors for infection rate and useful and practical clinical variables that can increase the pre-test probability of Chagas infection in low-resource settings.

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LONGITUDINAL CHANGES IN VECTOR BORNE DISEASE PREVALENCE IN A UNITED STATES DOG POPULATION

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Zoonotic vector-borne diseases, such as Lyme disease, have increased in prevalence in both animals and humans over the past decade. While these diseases spread due to various factors, little is understood about the progression of these diseases over time in animals with other co-morbidities, such as leishmaniasis. Within a cohort of approximately 600 dogs enrolled in a phase-III clinical trial of an experimental vaccine for leishmaniasis, a subset of 200 dogs were included in a longitudinal study to assess long term changes in ehrlichiosis, anaplasmosis, borreliosis, and heartworm infection status and to determine if there is a causal relationship between these comorbid infections and presentation with clinical leishmaniasis. The cohort included dogs from the Western, Midwestern, Eastern, and Southern regions of the United States. Dogs were tested by serology and PCR both pre- and post-primary vector season. Diagnostics used to assess these changes in vector borne diseases included the IDEXX SNAP® 4Dx® Plus Test and PCR to identify specific species of *Anaplasma*, *Ehrlichia*, *Borellia* and *Dirofilaria immitis* (dog filarial heartworm). At 6 months, a small geographic subset was also assessed for acute infection with *Anapaslma phagocytophilum*, *Anaplasma platys*, and *Ehrlichia canis*. All dogs were evaluated for clinical signs of erhlichiosis, anaplasmosis, Lyme disease, and heartworm at enrollment and at 6 months via physical examination. Additional information, including information regarding each dog's age, sex, *Leishmania* status (combination of PCR, serology, and clinical signs), and leishmaniasis vaccination status were assessed to determine how vector borne comorbidities affect progression of leishmaniasis in a dog population endemic for leishmaniasis. Initial results from trial enrollment indicated that 100% of dogs symptomatic for leishmaniasis and 65% of asymptomatic dogs had a co-infection with at least one of four other vector-borne diseases. This prospective cohort study will help to better understand the potential causal relationship between co-infection with vector borne diseases and progression of leishmaniasis.

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SERIAL EVALUATIONS OF THE PARASITE LOAD OF *LEISHMANIA (VIANNIA)* SPECIES BY QPCR (QUANTITATIVE POLYMERASE CHAIN REACTION) OF NATURAL INFECTED WILD AND SYNANTHROPIC RODENTS FROM AN ENDEMIC AREA OF SOUTH FOREST ZONE OF PERNAMBUCO, BRAZIL

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This study was undertaken to evaluate parasite levels of *Leishmania (Viannia*) species in wild and synanthropic rodents from an endemic American Cutaneous Leishmaniasis (ACL) foci in the South Forest Zone of Pernambuco, Brazil and assess their importance in the enzootic cycles. Wild and synanthropic rodents were captured tagged released and recaptured between May 2012 and August 2014. *L. (Viannia*) infections were evaluated by qPCR of skin and blood samples. Xenodiagnosis was performed with *Nyssomyia. whitmani* and *Lutzomyia longipalpis*. A total 603 rodents were marked with microchips. 40.6% (245/603) were *Nectomys squamipes*, 24.5% (148/603) were *Rattus rattus*,13.8% (83/603) were *Necromys lasiurus.* Of these 186 animals were monitored at 394 recaptures (Recapture 1 (R1) = 186, R2 = 97 R3 = 52 R4 = 27 R5 = 18, R6 = 6, R7 = 3, R8 = 2, R9 = 2 and R10 = 1). 29.2% (176/603) showed natural *L. (Viannia*) infections. The infection rates determined by qPCR were as follows*: N.squamipes* 43.8%, *N.lasiurus 30.1%, R.rattus* 16.2%, *Oxymycterus angulares* 16.3%, *Holochilus sciureus* 25%, *Akodon arviculoides* 9.4%. The infections in *Rattus rattus* – a synanthropic rodent – was 4.2%, 54.2% in plantations and 41.7% in houses. The only species recaptured 5 times was *N. squamipes*. The parasite load of rodents during recaptures (0- 50060.71 fg DNA) and remained high being highest during October and November 2012 and June 2013 and January 2014. Parasitamia fell in animals kept in the laboratory. There was no difference in xenodiagnoses positivity between the two sand fly species nor was there a positive association between parasite load and positive xenodiagnoses. In conclusion, the results reinforce the hypothesis that the enzootic is maintained in a mosaic rodent species and that parasitemia levels are maintained by re-infections. The presence of infected *R.rattus* in plantations and houses strongly supports peri-domestic transmission, especially as *Ny. whitmani* populations are highest in these niches. We consider that the detected infections were *L.(V.) braziliensis* as this species has previously been isolated from wild animals, sand flies and man in this very same area.

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ESTIMATING THE COSTS AND COST-EFFECTIVENESS OF EARLY DIAGNOSIS AND TREATMENT OF CHAGAS DISEASE IN COLOMBIA

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Chagas disease still remains an important public health problem in Latin America. Early stage treatments for curbing the progression of the disease have proved effective. However, access to Chagas disease diagnosis and treatment still remains very low in most endemic countries. In Colombia it is estimated that 5 million people are at risk of acquiring the infection, 436,000 people are already infected and 30% of these are prone to develop heart complications, yet access to diagnosis is estimated at <1% of the at-risk population. In Colombia the costs and cost-effectiveness of preventive and treatment strategies have not been quantified. We estimate the unit costs of (i) preventing Chagas by mass screening school-aged children and (ii) treating inpatient and outpatient episodes of those with Chagas (according to disease stage). Costs are presented in 2014 USD and based on (i) primary data collected from a provider perspective at national, departmental and municipality levels; (ii) in-depth interviews from Ministry of Health officials and municipal and departmental secretaries of Health in Boyacá, Casanare, and Santander and, (iii) a third-payer perspective, using the National Registry of Health Services between 2008 and 2014 for 7,227 patients. Having calculated unit cots as median and interquartile range (IQR), cost-effectiveness is modelled using the authors' previously developed burden of disease model, to estimate the incremental cost per DALY averted by scaling up mass screening. Early diagnosis was costed at $18 (IQR: 4-46) per school-child screened. Average treatment cost per patient per year was estimated at $29 (IQR: 15- 94) for mild Chagas and $211 (IQR: 104-541) for severe Chagas. On average a hospital admission for a patient with a primary diagnosis of Chagas cost $340 (IQR: 310-882, and maximum $16,685). However, outpatient clinic services, (doctor visits and ambulatory procedures) were the main cost driver, at 86% of total Chagas treatment costs. Preliminary modelling results suggest that mass screening for Chagas amongst school-aged children is cost-effective in a variety of epidemiological contexts.

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UNDERSTANDING LONG-TERM CYCLES OF VISCERAL LEISHMANIASIS IN BIHAR

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Visceral leishmaniasis (VL) is a vector-borne disease of public health importance in India, with the highest burden of disease in the states of Bihar, Jharkhand, West Bengal and Uttar Pradesh. VL is currently targeted for elimination by 2017; the primary interventions used for elimination are indoor residual spraying, active case detection and treatment. Historically the disease trend in India has been regarded as cyclical with case resurgence characteristically occurring every 15 years. However, the cause of this pattern remains unclear and as the Bihar VL programme nears the elimination target, explaining previous trends to use within predictive tools to avoid future epidemics has become essential. To interpret observed cyclical trends, annual climatic indicators including rainfall, temperature and humidity over time periods known to influence disease and vector trends over the year were compared with annual VL case incidence data. Rainfall was found to have a strong association with annual VL case patterns during the monsoon season (June to September) (p=0.0383) and prior to sand fly peaks (February to May, September and October) (p=0.0253). Whilst annual rainfall was also found to have a close association with annual case incidence (p=0.0398), rainfall during the sand fly peaks (March to June, October and November) was not significant (p=0.0911). No association between humidity or temperature and VL incidence was detected (all values p=>0.05). The VL programme in Bihar has made significant progress in recent years, adopting improved practices for vector control, diagnostics and treatment strategies: changing from DDT and stirrup pumps to alpha-cypermethrin and hand compression pumps, introduction of the rk39 test and wide-scale availability of amphotericin B. Such concerted efforts may lead to short term success, however to achieve and sustain elimination, a better understanding of external causative factors is required.

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CHAGAS DISEASE PREVALENCE AND RISK FACTORS IN WORKING DOGS ALONG THE TEXAS-MEXICO BORDER

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Chagas disease is a neglected tropical disease caused by the protozoan parasite *Trypanosoma cruzi* and transmitted by hematophagous triatomine vectors. Chagas disease is estimated to affect 6 million people throughout Latin America and is increasingly recognized in humans and dogs across the southern U.S., where studies have found that exposure of shelter and stray dogs ranges from 3.6-22.1%. Our objective was to assess the prevalence and distribution of canine Chagas disease in dogs along the Texas-Mexico border, a suspected focus for local transmission of the parasite. Department of Homeland Security (DHS) working dogs play important security roles including detection of narcotics and concealed humans, and may be at high risk due to prolonged work outdoors in borderland regions with established kissing bug populations. From fall 2015 to summer 2016, we collected blood samples from dogs in five different geographical management areas, including dogs along the geopolitical border as well as north of the border. Canine plasma was screened for anti-*T. cruzi* antibodies by rapid immunochromatographic serological tests, and positivity was confirmed by indirect fluorescent antibody testing and an independent immunochromotagraphic assay. To test for active infection, buffy coat samples were tested by qPCR to amplify *T. cruzi* satellite DNA. The preliminary results (n=528) indicate over 11.9% [9.2, 14.7] of dogs are positive for anti-*T. cruzi* antibodies by two or more serology assays, and parasite DNA was detected in three blood samples. Seropositivity did not differ across age, sex, breed, geographic location or canine discipline. Ongoing work aims to track canine infection over time in relation to clinical status and vector occurrence. Understanding the epidemiology of Chagas disease along the border is a prerequisite for implementing vector control measures to protect the health of not only these high value working dogs, but also local human populations.

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EPIDEMIOLOGICAL EVIDENCE OF CANINE VISCERAL LEISHMANIASIS IN IÑAPARI-PUERTO MALDONADO, PERU, BORDER WITH BRAZIL AND BOLIVIA

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Visceral Leishmaniasis (VL) is lethal when it is not detected and treated promtly. In Latin America, it is caused by *Leishmania (L.) chagasi* being endemic in 11 countries such as Colombia, Bolivia and Brazil, neighbouring countries of Peru, where still not reported cases of LV. The aim of this study was to verify the presence of epidemiological risk factors involved in the transmission of LV at Iñapari locations, border district with Brazil and Bolivia. An exploratory cross-sectional descriptive study was conducted in two stages: i) Serological search using the Inmunocromatográfico Dual Path Platform and Indirect Immunofluorescence tests, to detect specific

antibodies against *L. (L) chagasi* in canine population of: a) Iñapari, urban locality b) Villa Primavera, rural location, c) Belgium, native rural location; ii) entomological survey with CDC traps and taxonomic identification with Young & Duncan's keys. Data and clinical signs of dogs were recorded in a Veterinary Protocol Field. We sampled 134 dogs, detecting specific antibodies against I in 7/134 (5.22%) of the population in study; the percentage distribution by localities was 2/28 (7.14%) for Belgium, place where it was observed a close coexistence between the settlers and their similar Brazilian beyond right of the Acre River; 4/87 (4.6%) for Iñapari and 1/19 (5.26%) in Villa Primavera; general clinical condition was fair to poor with eczematous pictures, alopesia and oncinogrifosis in 12% of dogs. Sampling of sand flies were captured in the three locations highlighting the identification of *Lutzomyia nevesi,* belonging to *Lu verrucarum* group, near *Lu. evansi,* vector of LV. This is the first study of LV performed in Peru, in which we bring a prevalence of 5.22% for canine visceral leishmaniasis in Iñapari, district border with Brazil and Bolivia; this finding, is significant because up to now Peru is considered to be a country free of LV. Therefore it is recommended to implement epidemiological surveillance studies in search of the vector and the etiologic agent of the LV in high risk living borders in Peru, in order to control the spread of LV into new geographic areas in Latin America.

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THE IMPACT OF TEN-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV10) ON *STREPTOCOCCUS PNEUMONIAE* NASOPHARYNGEAL CARRIAGE RATE: PHENOTYPIC AND GENETIC DIVERSITY OF ISOLATES FROM VACCINATED CHILDREN IN ADDIS ABABA, ETHIOPIA

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Streptococcus pneumoniae (*Pneumococcus*) among the most important human pathogens, with high morbidity and mortality rates. Nasopharyngeal colonisation is the necessary first step in the pathogenesis of associated invasive pnuemococcal diseases. Ethiopia, introduced the ten-valent pneumococal conjugate vaccine (PCV110) since October, 2011, there is nevertheless lack of adequate baseline information on epidemiological factors for subsequent impact assessment. The aim of this study was to determine phenotypic and genotypic diversity nasopharyngeal isolates of *Streptococcus pneumoniae*. A total of 789 newborn babies were enrolled at the age of six weeks when they came for the first PCV10 vaccine, and 206 were re-sampled at the age of nine months and 201 at two years after final dose of PCV10. Nasopharyngeal swabs were taken for bacteriological analysis before vaccination at the age of six weeks, and after completion at the age of nine months and two years. Isolates were tested for commonly used antibiotics by disc diffusion method and those that isolates showed resistance for penicillin and erythromycin the minimum inhibition concentration were determined by E-test A total of 325 pneumococcal isolates were serotyped and characterized by Pulsed Field Gel Electrophoresis and 12 isolates were analyzed by multilocus sequence typing. The carriage rate of *S. pneumoniae* at the age of six weeks, nine months and two years was 26.6%, 56.8% and 47.6% respectively. A total of 61 serotypes of *S. pneumoniae* were identified from 325 isolates, and 6A, 11A, 15B, 23F,15A and 19F dominated in decreasing order. The proportion of serotypes covered by PCV10 vaccine among the isolates at 6 weeks and 9 months were 20.2% and 11.1% respectively. Molecular typing further showed a presence of high genetic diversity.The antibiotic test indicated that resistance rates were ranging from 4.3% for chloramphenicol to 27.7% for Trimethoprim/sulfamethoxazole. In conclusion, this study highlights the presence of very diverse serotypes in the country, and PFGE and MLST result indicates case of a possible capsular switching event.

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ASSESSING THE BURDEN OF STIGMA AMONG TUBERCULOSIS PATIENTS IN A PASTORALIST COMMUNITY IN KENYA

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Tuberculosis (TB) epidemic is one of the important global humanitarian and development challenges. Stigma associated with TB has been termed as a barrier to prompt diagnosis and treatment compliance. Although TB stigma is recognised as a serious problem, it has been difficult to describe the magnitude and therefore the public health importance of the problem due to lack of quantitative measures of stigma in the African context. Due to lack of an instrument to measure TB stigma in Kenya at the time of our study, we examined the adaptability of the TB stigma scales previously developed and validated in Thailand. The purpose of this study was to assess and quantitatively measure TB-related stigma as well as identify factors associated with it among patients in rural Kenya. This was a mixed method study. Data were collected using questionnaires, four focus group discussions and ten patient's narratives. A questionnaire containing socio-demographic characteristics and scales measuring perceived TB stigma and experienced/felt TB stigma, was administered to 220 patients on TB treatment in the period between July-December 2015. Assessment of psychometric properties of the scales included basic statistical tests, evaluation of Cronbach's alpha and factor analysis. Multiple linear regressions were performed to determine factors associated with higher TB stigma scores. The study showed that internal consistency reliability coefficients were satisfactory with Cronbach alphas of 0.87 and 0.86 for the 11-item and 12-item scale. The investigation revealed that experienced TB stigma was high and symptoms similar to those of AIDS, as well as fear of infection through casual contact, such as eating with friends and touching others were significant determinants of TB stigma. Low level of education (mean difference of 1.85; 95% CI: 0.09, 3.62) and female gender (mean difference of 3.61; 95% CI: 2.11, 5.11) were significantly associated with higher stigma scores while age, marital status, occupation and the patient's religion were not. There is need to implement stigma reduction interventions to mitigate the impact of stigma and improve TB program outcomes.

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PREVALENCE OF PNEUMOCOCCAL CARRIAGE AND ANTIMICROBIAL SUSCEPTIBILITY AMONG CHILDREN TARGETED BY VACCINATIONS PROGRAM IN BURKINA FASO

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In Western Africa *Pneumococcus* is the most common pathogen isolated in purulent meningitis. In Burkina Faso the fatality rate remains high (up to 46%) among *Pneumococcus* infected patients treated with Penicillin or ceftriaxone which are the most common drugs used in this country. This study aims to describe the prevalence of pneumococcal carriage and antimicrobial susceptibility among children targeted by pneumococcal vaccinations program in Burkina Faso. The data were collected during the baseline assessment of an interventional cluster randomized controlled study (SMC-AZ project) evaluating the impact of Sulfadoxine-Pyrimethamine (SP)+Amodiaquine (AQ) combined with azithromycin (Z) on children mortality rate years in the district of Hounde (located in the Western Region of Burkina Faso). In August 2014 nasopharyngeal swabs were collected among 430 children aged 0-5 years old before they have been randomized to receive either "Sulfadoxine-Pyrimethamine

(SP) + Amodiaquine (AQ) combined with azithromycin (Z)" or "SP + AQ + placebo". We used pneumococcal positive cultures to determine resistance profile. The pneumococcal identification was made from morphology and conventional characterization methods. (Azythromycin, Oxacillin, Ceftriaxone, Norfloxacin, Vancomycin, Gentamicin, Erythromycin. Resistance to penicillin and macrolides will be confirmed by E-test strips. Among 430 nasopharyngeal specimens collected, 189 (43.96 %) had positive cultures of pneumococcus. The prevalence of pneumococcal resistant strains was 26.46 % (16/189) and 1.06% (2/189) for penicillin and ceftriaxone respectively: This resistant prevalence for azithromycin was 4.23% (8/189). In conclusion, due to the emergence of penicillin resistance as shown by our study, ceftriaxone becomes the most suitable antibiotic for the treatment of pneumococcal infections. However the presence of Pneumococcal strains resistant to ceftriaxone highlights the need to closely monitor this cephalosporin antibiotic.

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EFFECT OF OVEREXPRESSION OF *MYCOBACTERIUM TUBERCULOSIS* RPSA PROTEIN IN MOLECULAR MECHANISM OF RESISTANCE TO PYRAZINAMIDE IN *M. SMEGMATIS*

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Tuberculosis remains a major cause of illness and death worldwide causing 1.6 millions of deaths annually, being exacerbated by the epidemic co-infection of HIV-TB and the emergence of multidrug-resistant strains and extremely resistant in developing countries like Peru. Pyrazinamide (PZA) is one of the most important drugs used in the combined anti-tuberculosis therapy, drug that is used as a first option to treat Tuberculosis. After the drug enters *Mycobacterium tuberculosis* (MTB) is hydrolyzed by the pyrazinamidase to the bactericidal molecule, pyrazinoic acid (POA). A recent study identified a target of pyrazinoic acid, ribosomal protein S1 (RpsA). RpsA is a protein involved in the process ribosomal trans-translation, which has been associated with bacterial survival in stress conditions, nutrient starvation and virulence. Despite its importance, some of PZA action mechanism involved are still poorly understood. *M. smegmatis* presents highly POA active efflux pumps, 900 times faster than *M. tuberculosis*, largely explaining part of their natural resistance to PZA. To further understand *M. smegmatis* PZA action mechanism, we evaluate the role of *M. tuberculosis*-RpsA overexpression in *M. smegmatis*. To evaluate this effect, *M. tuberculosis*-RpsA recombinant protein was expressed in *M. smegmatis* system, followed by a PZA- drug susceptibility test using the minimum inhibitory concentration. Our results showed a phenotypic change from naturally resistant *M. smegmatis* (MIC: > 15 mg/ml) to sensitive (MIC: 0.468 mg/ml). This evidence suggests that *M. tuberculosis*-RpsA proteins, which are part of ribosomes might be binding POA in *M. smegmatis*, therefore inhibiting trans-translation system and its viability.

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TB OR NOT TB? A MODEL FOR INTEGRATING PARAGONIMIASIS SURVEILLANCE AND CONTROL WITH TUBERCULOSIS CONTROL PROGRAM

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Misdiagnosis of paragonimiasis as pulmonary tuberculosis (TB) due to similar clinical manifestations results in continuing morbidity and loss of productivity. Integration of surveillance and control of paragonimiasis with the TB control program may be important especially in co-endemic areas to prevent misdiagnosis. This study aimed to describe the prevalence of paragonimiasis, TB, and coinfections in six municipalities in Zamboanga Region, Philippines using a model for integrating paragonimiasis surveillance and control with tuberculosis control program, as well as to analyze the cost of implementing the aforementioned model. Active surveillance for TB and paragonimiasis was conducted in nine barangay clusters, while passive surveillance was implemented in two rural health units (RHUs) for at least three months. A simple cost analysis compared the cost of implementing the model with the National Tuberculosis Control Program (NTP). Four hundred patients were included in the active surveillance, seven of whom had paragonimiasis (2%), while three had TB (1%). Out of the 54 patients included in the passive surveillance, one (2%) had paragonimiasis. A simple cost analysis showed that the marginal cost of implementing the model is lower than the average cost of implementing the NTP. The study showed that the integration of surveillance and control of paragonimiasis with the TB control program is feasible and contributed in describing new paragonimiasis foci, as well as finding and treating misdiagnosed paragonimiasis and TB cases. Optimization of the model and scaling up of its implementation are recommended prior to its proposed integration with NTP.

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TUBERCULOSIS OUTBREAK INVESTIGATION IN A COLONY OF *AOTUS* MONKEYS: DIAGNOSIS, EPIDEMIOLOGY AND CROSS-SECTIONAL RANDOMIZED SCREENING USING ANTIBODY AND WHOLE-BLOOD *IN VITRO* INTERFERON-γ RELEASE TESTING

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Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (MTB) is a devastating and terminal disease in non-human primates (NHPs). Regular TB screenings using the intradermal tuberculin test (TST), despite its low specificity, have been the mainstay of TB surveillance and control in NHPs. However, the lack of a reliable source of old tuberculin has hampered TB screening programs in NHP colonies around the world. Historically, *Aotus* monkeys have been considered less susceptible to TB than Old-World NHPs. Here we present the diagnosis and epidemiology of a TB outbreak in a colony of ~400 *Aotus* monkeys at The Gorgas Memorial Institute in Panama during the first half of 2015 that killed 7 animals and the results of two cross-sectional randomized TB screening studies, using antibody (Ab) and IFN- γ release assay based testing, eight years apart. The outbreak started on January 6th, 2015, with the death of a lab-bred 9 year-old 600 g male splenectomized *Aotus* that died with signs of a chronic wasting disease (index case). *M. kanssasi* was isolated from a lung tubercle of this animal. Between January-June, 2015, six additional TB cases occurred, three confirmed with MTB isolation and three suspicious by histopathology. Control measures included, quarantine, disinfection and TST screening of all personnel. In the Ab based screening study of 2008, only one animal out of 50 tested, reacted weakly to the Immunochromatographic PrimaTB STAT PAK® assay. This reaction was considered a false positive. In the second study in 2016, 34/34 animals of a total adult population of 313 *Aotus* resulted negative in the Primagam® IFN-γ release assay. TST screening was negative in all animal handlers after the outbreak. The source of infection was not identified, though human to monkey transmission is suspected. Genotyping of MTB isolates and a screening program based on the Primagam® IFN- γ release assay are underway. This is considered to be the first *Aotus* TB outbreak reported at the Gorgas Memorial Institute *Aotus* monkey colony since its inception in 1976.

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DETERMINATION OF PLASMA LEVELS OF LEVOFLOXACIN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR USE AT A MULTIDRUG-RESISTANT TUBERCULOSIS HOSPITAL IN TANZANIA

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Therapeutic drug monitoring may improve multidrug-resistant tuberculosis (MDR-TB) treatment outcomes. Levofloxacin demonstrates significant individual pharmacokinetic variability. Thus, we sought to develop and validate a high-performance liquid chromatography (HPLC) method with ultraviolet (UV) detection for levofloxacin in patients on MDR-TB treatment. The HPLC-UV method is based on a solid phase extraction and a direct injection into the HPLC system. Human plasma was loaded onto Oasis SPE cartridges, conditioned, washed and eluted. The assay parameters of accuracy, precision, recovery and limits of quantification were determined using human plasma spiked with known concentrations of levofloxacin. This method was then utilized to measure levofloxacin concentrations from patients' plasma samples from a retrospective cohort of consecutive enrolled subjects treated for MDR-TB at the national TB hospital in Tanzania during 5/3/2013- 8/31/2015. Plasma was collected at 2 hours after levofloxacin administration, the time of estimated peak concentration (eCmax), after 2 and 4 weeks of treatment. Forty-one MDR-TB patients had plasma available and 39 had traceable programmatic outcomes. Only 13 (32%) patients had any plasma concentration that reached the lower range of the expected literature derived Cmax of 8 µg/mL. In patients with an eCmax ≥ 7.0 µg/mL compared to those with eCmax <7.0 µg/mL, the time to sputum culture conversion was 37.6 ± 22.1 days vs. 48.7 ± 26.8 days (p=0.19) but a trend was observed in greater proportion of cure in 10 out of 17 (58.8%) vs. 6 out of 222 (27.3%) (p=0.05). Furthermore, one patient with an eCmax/minimum inhibitory concentration (MIC) of only 1.13 µg/ml acquired extensively drug resistant (XDR)-TB while undergoing treatment. The HPLC-UV methodology for determination of levofloxacin concentrations achieved excellent accuracy and reproducibility along a clinically meaningful range. The individual variability of levofloxacin concentrations in MDR-TB patients from Tanzania supports further study of the application of onsite therapeutic drug monitoring and MIC testing.

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MOLECULAR CHARACTERIZATION OF CIRCULATING STRAINS OF INFLUENZA A BETWEEN 2014-2015 IN EGYPT

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Influenza viruses are continuously evolving with the potential for new subclades with altered antigenicity. Hence, tracking genetic changes is crucial for selection of effective vaccine strains, detection of drug resistance and determination of virulence markers. Herein, we analyzed the hemagglutinin (HA) and neuraminidase (NA) genes of influenza A/ H3N2 and H1N1 pdm09 circulating in Egypt during the winter of 2014/15. Patients were considered to have influenza-like illness (ILI) if they met the WHO criteria. Oropharyngeal swabs in viral transport media collected from ILI patients from seven sites within Egypt were tested by real-time PCR to determine influenza subtypes. Positive influenza A samples were inoculated in MDCK cells and representative isolates chosen for HA and NA sequencing. Phylogenetic analysis of the HA gene obtained from 13 H3N2 viruses showed that nine clustered within genetic subgroup 3C.2a, and four clustered within clade 3C.3 with three in subgroup 3C.3b. All sequences showed 97.6 - 98.3% nt similarity to clade 3C.1 vaccine strain of 2014/15 season (A/Texas/50/2012), and 98.4 - 98.9% nt similarity to clade 3C.3a 2015/16 candidate vaccine strain (A/Switzerland/9715293/2013). Of note, viruses in which HA genes clustered within subgroup 3C.3b had three distinct mutations in their NA protein (Y155F, D251V, and S315G) that define a distinct cluster with other recently collected viruses. The HA gene sequences obtained from four Influenza A H1N1 isolates clustered within clade 6B, with a notable observation in one sequence of a D222G mutation previously reported to be associated with severe disease outcome. The NA gene sequences of A/H3N2 and H1N1did not reveal mutations previously reported to be associated with resistance to NA inhibitors. Sequence analysis results of influenza A/H3N2 revealed that more than 60% of sequences clustered within the 2014/15 3C.2a subgroup. Clade 6C clustering was not observed among A/H1N1 viruses. Representative influenza A strains analyzed during this season remain susceptible to NA inhibitors. We report the first observance of a severe disease marker (D222G) among H1N1 viruses.

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UNDERSTANDING THE IMMUNE RESPONSE TO *STREPTOCOCCUS PNEUMONIAE* FROM VACCINATION AND CARRIAGE ON A PROTEOME SCALE

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Antigenic diversity presents a challenge for pneumococcal vaccine development. Technological limitations have hindered characterization of immune responses to the diverse protein repertoires observed within pneumococcal populations. Proteome microarrays permit exploration of the antibody response against entire proteomes. We profiled IgG responses against a *S. pneumoniae* (*Sp*) whole cell vaccine (SPWCV) in a Phase I trial with 42 U.S. adult participants, and against nasopharyngeal colonization in a longitudinally-sampled birth cohort of 63 infants residing in the Maela refugee camp near the Thailand-Myanmar border under natural exposure to pneumococci. We used the TIGR4 core proteome and 90 isolates from Massachusetts, United States to construct a "pan-genomic" *Sp* whole proteome microarray and probed serum samples. Large differences in the IgG response to Sp array proteins were reproducible between individuals. Protein traits significantly associated with elevated immunogenicity included increased length, signal peptides for secretion, and cell surface attachment domains. IgG responses to 166 unique *Sp* proteins increased after vaccination with SPWCV, and a dose response was observed. In the Maela infant cohort, the antibody kinetic profile against hundreds of *Sp* proteins followed a trend of sharply declining antibody levels between birth and 6 months of life, followed by a gradual increase during the following 18 months of follow-up. Both variable antigens, including zinc metalloproteases and choline binding proteins, and more conserved peptidoglycan synthesis machinery and transporters elicited high IgG responses. Proteome microarrays can facilitate vaccine development and test hypotheses relating to bacterial evolution, population genetics and epidemiology.

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EXPANSION AND EVALUATION OF TUBERCULOSIS MICROSCOPIC-OBSERVATION DRUG-SUSCEPTIBILITY ASSAY (TB-MODS) IN EGYPT, 2014-2015

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Microscopic-Observation Drug-Susceptibility (TB-MODS) assay is a liquid culture-based test that detects *Mycobacterium tuberculosis* and assesses isoniazid (INH) and rifampicin (RIF) resistance directly from sputum samples. Like Mycobacteria Growth Indicator Tube (MGIT) culture, the method markedly reduces growth time compared to solid media and enables detection of multidrug-resistant tuberculosis (MDR TB). However, TB-MODS is less expensive and has produced faster results than MGIT in low-resource settings. Our objectives were to implement TB-MODS in Egypt for routine TB diagnosis and treatment decisions, compare it to MGIT, and expand its use in high-TB burden governorates. Between October 2014 and September 2015, TB-MODS was implemented at the central and three high-TB burden governorate laboratories in Egypt where MGIT culture and drug susceptibility testing (DST) are used. Test characteristics of TB-MODS were compared to MGIT using the results of sputum samples from presumptive TB patients. There were 521 sputum samples cultured by both TB-MODS and MGIT techniques. Compared to MGIT, the sensitivity and specificity of TB-MODS in detecting TB was 99.5 % and 97.1%, respectfully. The time from specimen processing to DST results was 11.3+6 days for TB-MODS and 18.9+13.9 days for MGIT ($p<0.01$). The proportion of samples tested by TB-MODS with cultures resistant to INH was 31.0% (118/381), RIF was 28.1% (107/381), and both (i.e. MDR TB) was 22.9% (86/376). Compared to MGIT, the sensitivity and specificity of TB-MODS in detecting resistance to INH was 86.8% and 94.9%, to RIF was 86.5% and 95.9%, and to both drugs was 88.1% and 95.9%, respectfully. TB-MODS appears to be a reliable and more rapid alternative to MGIT for detecting TB and performing first-line DST, including for MDR TB, in Egypt.

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THE SPATIAL-TEMPORAL DISTRIBUTION OF *ONCOMELANIA HUPENSIS* ALONG YANGTZE RIVER AFTER IMPLEMENTATION OF AN INTEGRATED CONTROL STRATEGY IN JIANGSU, P.R. CHINA

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Using spatial-temporal analysis to explore the distribution and of *Oncomelania hupensis*, the intermediate host of *Schistosoma japonicum*, along Yangtze River under an integrated control strategy in Jiangsu, P.R.China,. The density and spatial location of live and infected snails from 2001 to 2013 were collected in fields. Descriptive analysis and mapping were respectively used to detect the changes and distribution of live and infected snail in different years. Global and local spatial autocorrelation analysis were carried out to find the trend and area of spatial cluster at different years. The spatial-temporal scan analysis was used to identify the risk area and temporal during the study period. The number and area of habitats, densities of live and infected snails were increasing before 2004, then went into a rapid decreasing after implementation of an integrated control strategy, and went into a relatively slow declining after 2009. The distribution map showed the number of high density habitats was declining, and the location was transferring from west to east. Global spatial autocorrelation showed there were spatial clustering of live and infected snails when the density was relatively high at the province scale. Local spatial autocorrelation revealed that the number of specific clustering area were declining and transferred to the middle reaches of Jiangsu province. Two high risk area of live snails located in upper and middle reaches of Yangtze river, and one high risk area of infected snail located in upper reach. In conclusion, the integrated control strategy was more effective in Jiangsu province. Next, more control resource should be settled in the middle of Yangtze river, Jiangsu.

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THE SEROLOGICAL DIAGNOSIS METHODS OF SCHISTOSOMIASIS AT DIFFERENT PREVALENCE: A META-ANALYSIS

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Use meta-analysis of diagnostic tests to comprehensive evaluation of indirect hemagglutination test (IHA), enzyme-linked immunosorbent assay (ELISA) and dipstick dye method (DDIA) in the diagnosis of schistosomiasis japonica at different prevalence. Through literature reveiw according with the inclusion and exclusion criteria to establish a database, and use Meta-disc and R software to make Meta-analysis of threshold test, heterogeneity test, weighted by the quantitative effect of merger and SROC curve fitting, etc. Results A total of 84 papers were included in the final analysis. The sensitivity of IHA respectively were 0.84, 0.76 and 0.94 in heavy, medium and low endemic areas, and specificity were 0.73, 0.64 and 0.73; sensitivity of ELISA respectively were 0.88, 0.80 and 0.93 in heavy, medium and low endemic areas, and specificity were 0.59, 0.59 and 0.62; sensitivity of DDIA respectively were 0.93, 0.81 and 0.93 in heavy, medium and low endemic areas, and specificity were 0.66, 0.69 and 0.59. Weighted sensitivity of IHA, ELISA and DDIA were 0.83, 0.87 and 0.90; The weighted specificity was 0.69, 0.60 and 0.62. The areas under the curve of SROC respectively were 0.89, 0.96 and 0.92 in the IHA, ELISA and DDIA. In conclusion, in different prevalence, there is some differences effectiveness of methods for serological diagnosis of schistosomiasis. The method of IHA, DDIA and ELISA is relatively high effectiveness in low, moderate and severe endemic areas, respectively. The sensitivity and specificity of all diagnostic methods of schistosomiasis are required further improved.

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A PILOT STUDY ON HUMAN SCHISTOSOMIASIS IN A RURAL COMMUNITY OF FIANGA, REPUBLIC OF CHAD

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Schistosomiasis, a water-associated parasitic disease and part of neglected tropical diseases (NTDs), still poses a significant public health threat in many part of Africa. In recent years there have been increasing national control programs against schistosomiasis and other NTDs. This was encouraged by the World Health Assembly resolution WHA 54.19. Human schistosomiasis is an important public health problem in Cameroon and Chad but the control program is only implemented in Cameroon. Dacheka (Cameroon) and Fianga (Chad) are 2 neighboring sub-divisions located across the border of the 2 countries. People from both sides constantly move across the border and this can affect the epidemiology of schistosomiasis in both sides. The aim of this study is to assess current schistosomiasis situation in villages in the Chadian side and how it might impact the situation in Cameroon. Here we present preliminary results concerning the parasitological survey and environmental characterization on potential transmission sites. This study was conducted in December 2014 and 5 schools were selected in Fianga, based on the geographic localisation. In each school, 50 schools children were randomly selected to be part of the survey. Upon receiving approval of parents and local authorities, selected children were asked to provide stool and urine specimens which were examined using the Kato-Katz and sedimentation techniques, respectively. Overall, only *Schistosoma haematobium* eggs were found in urine samples: prevalence of infection of 53.4% ±0.5 and an average of 13±23 eggs/20µl of sediment. Prevalence of infections in Kiriou, Tchangbele and Deheing were ≥ 50% whereas in Gabra and Kaski they were between 10% and 49%. From our investigation we observed

that villages were very poor and relied mostly on temporary water bodies for their daily activities. In kiriou, population create natural reservoirs to retain rainfall water during rainy period to help cultivation during the dry season. Urinary schistosomiasis is a real public health problem in Fianga and poor life and environmental condition seem to be important factors to be considered for future control plan.

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EFFICACY AND DRUG ACTION MECHANISM OF ARTESUNATE AND A SYNTHETIC ENDOPEROXIDE COMPOUND N-89, AGAINST ADULT STAGE *SCHISTOSOMA MANSONI*

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Treatment and morbidity control of schistosomiasis largely relies on a single drug, praziquantel (PZQ), thus creating concerns about the selection of resistant worms due to repeated therapy. PZQ is also known to kill only adult *Schistosoma* worms and shows inability to abort early infection or prevent re-infection and its lack of prophylactic effect demands the need for novel drugs. N-89 a synthetic compound based on the endoperoxide structure of artemisinin has been previously shown to have anti-schistosomicidal effects against *S. mansoni* in both larvae stages and adult stages. In a murine model infected with *S. mansoni*, oral administration of 300 mg/kg of N-89 and artesunate showed significant worm burden reduction, hepatomegaly reduction and inhibiting granuloma formation at 2 weeks post-infection and a significant reduction in fecundity, egg burden and a significant reduction in body length for the N-89 treated group at 5 weeks post-infection. Scanning electron microscopy results revealed no tergumental damages suggesting that the drug targets may be internal. *In vitro* assessment of the survivability of *S. mansoni* worms cultured with 50 µM to 6.25 µM concentrations in 2-fold dilutions of N-89 and N-251 (a derivative of N-89) revealed IC_{50} values of 6.1±3.6 µM and 10.72±2.5 µM respectively. Artesunate however did not have any effects *in vitro* at the same concentrations. These results suggest that N-89 and artesunate may be possible drugs for schistosomiasis. This study therefore explores the exact biochemical action mechanism related to worm killing and fecundity reduction using molecular and immune-chemical techniques.

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DRUG DISCOVERY: *IN VITRO* EVALUATION OF EXTRACTS FROM MEDICINAL PLANT *BALANITES AEGYPTIACA* (LINN) DEL FOR ANTI-*SCHISTOSOMA* CERCARIAL PROPERTIES

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Schistosomiasis is a parasitic disease caused by schistosomes which manifests mainly in two forms; intestinal schistosomiasis caused by *Schistosoma mansoni*, *S. japonicum*, *S. intercalatum* and *S. mekongi* which is associated with bloody stool, and urinary (or urogenital) schistosomiasis caused by only *S. haematobium* with by bloody urine. Schistosomiasis continues to persist in many endemic communities with at least 261 million people need treatment in 2013. Successful control effect would largely depend on prevention of cercarial penetration of the human skin at the point of water contact. There is scarce information on preventive drug that is topically applied to prevent schistosomal cercaria from penetrating the human skin. Medicinal plants offer unique platforms for novel drug discoveries for treatment and prevention of many diseases including Neglected Tropical Diseases. The aim of this work was to screen medicinal plant extracts for anti-schistosomal cercaria activities. Aqueous (Aq), 70% (70EtoH) and absolute ethanol (Abs) extracts were prepared from *Balanites aegyptiaca* stem-bark and tested against cercariae at concentrations of 1000 to 10,000 ppm in 24-well culture plate at room temperature. The cytotoxic activities of the extracts on CHANG, PC-3 and MCF-7 cell lines were assessed by MTT assay. The Aq at 10,000 ppm demonstrated high anti-cercarial activity by killing the cercariae (20 to 80/test) within 7 mins and up to 36 mins at 1,000 ppm. However, the cercariae incubated with deionized water only had a mortality rate of 10.4% even after 240 mins. The Abs extract had IC50 values of >1000 µg/ml on PC-3 with 31.76 and 26.57 on MCF-7 and CHANG cells respectively. While the 70EtoH recorded 40.01, 60.80 and 64.25 against PC-3, MCF-7 and CHANG cells respectively. The IC50 for Aq were 44.96, 40.90 and 32.45 accordingly. While the curcumin control had 4.69, 15.22 and 11.67 against PC-3, MCF-7 and CHANG cells respectively. Extracts showed promising anti-cercarial activities that can be explored further for development of novel anti-cercarial drug that could be topically applied on the skin of population at risk to prevent cercaria penetration.

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OUTBREAK OF URINARY SCHISTOSOMIASIS IN A SCHOOL FOR MIGRANT CHILDREN, GYALLESU WARD, ZARIA, KADUNA STATE, NIGERIA. JANUARY 1ST-MAY 15TH, 2015

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Urinary schistosomiasis is a disease of poverty that leads to chronic ill health more than 700 million people live in endemic areas worldwide. The disease infects 240 million people globally with over 50% in African countries. School age children in regions with poor sanitation and contact with fresh water constitute the majority of cases in endemic areas of Nigeria. Urine mapping using rates of blood in urine of school age children showed multiple areas of endemicity in Kaduna state. We investigated to identify the cause of heamaturia among students and the associated risk factors. A descriptive study and a 1:1 unmatched case control study with 100 respondents were conducted to identify the source of infection. Cases were students with a history of visible heamaturia or with possible reagent slip for heamaturia residing in the school hostel, 1st January-15th May 2015. Ten Urine specimens and two water samples from the Galma River were sent for laboratory testing. We identified 50 cases (Overall attack rate 13.3%, CFR: 0%). Male: female ratio (1:0) between January-June, 2015. Mean age of cases was 10.2 years ± 1.4 years; mean age of controls 10.0 years ± 1.3 years. The attack rate was highest among 10-13 year old (35%). Compared with controls, the cases did not differ in terms of state of origin and environmental exposure. Cases were more likely to Swim in the river (odds ratio: 36; 95% CI: 7.3-241.4) and wash with contaminated water (OR: 7.4; 95% CI: 2.8-20.0). 30% of 10 Urine specimens tested positive for Ova, 50% of 2 water samples from Galma River were positive for cercariae of *Schistosoma haematobium*. The outbreak was confirmed to be Urinary schistosomiasis. The risk factors associated with the outbreak were: Swimming in the river and washing with contaminated water. We provided mass chemotherapy with Praziquantel and albendaxole, health education using community health teams and facilitated provision of portable water to the school. Findings will be used in mapping endemic areas for planned mass chemoprophylaxis, disseminated to stakeholders for use in planning public health policy and utilized for health education to populations at risk.

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ASSESSMENT OF THREE SCHISTOSOMIASIS ENDEMIC AREAS USING KATO-KATZ TECHNIQUE AND ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) ANTIGEN AND ANTIBODY TESTS

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Schistosomiasis is endemic in 12 out of the 16 administrative regions of the Philippines and undertaking surveillance to monitor endemic areas is necessary. The Department of Health (DOH) Philippines currently undertakes surveillance through parasitological examination using Kato-Katz technique. However, in areas where there is low level of endemicity, case detection through Kato-Katz technique may pose a major challenge due to the low sensitivity of the test. This study aimed to determine the prevalence of schistosomiasis in selected schistosomiasis-endemic provinces using Kato Katz technique and ELISA Antigen (Ag) and Antibody (Ab) tests. Areas identified as endemic and near elimination level for schistosomiasis were purposively selected. School-based collection of stool and blood samples was conducted and samples were examined using Kato-Katz technique and ELISA Ag and Ab tests, respectively. Results showed zero prevalence of schistosomiasis in Davao City, 0.5% in Davao del Sur, and 3.6% in Compostela Valley using Kato-Katz technique. Higher prevalences of schistosomiasis were observed for Davao City, Davao del Sur, and Compostela Valley with 5.0% and 34.4%; 3.0% and 19.2%; and 14.4% and 56.5% using ELISA Ag and Ab tests, respectively. Results of the study showed that the use of Kato-Katz technique in highly endemic areas is still helpful in diagnosis of infected individuals. In low endemic areas, surveillance of schistosomiasis using ELISA Ab test may provide a better evaluation of the transmission status of the infection at population level necessary in the policy formulation for appropriate surveillance and implementation of control measures. ELISA Ag test, on the other hand, may provide more accurate diagnosis of the infection in low transmission areas necessary in the treatment of the infection that could contribute to the control of transmission of the infection in the community. Further studies are needed to support the use of these diagnostic techniques in a stratification scheme to be utilized by Schistosomiasis Control and Elimination Programs in light of the other strategies being implemented at the community level.

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DETERMINING THE IMPACT OF *SCHISTOSOMA MANSONI* INFECTION ON PUBLIC HEALTH IN A HIGH PREVALENCE REGION IN REMOTE MADAGASCAR

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Schistosomiasis is widespread in Madagascar but many treatment campaigns are unable to reach some of the more rural and remote regions of the country. The aims of our research expeditions were to determine the prevalence and level of disease burden of schistosomiasis in the Marolambo district of Eastern Madagascar (one of Madagascar's most remote regions), to provide treatment for schistosomiasis and initiate a health education program. We screened school aged children (five to fourteen years of age) for schistosomiasis from six schools along the Nosivolo River in Marolambo, using circulating cathodic antigen (CCA) testing and Kato-Katz procedures. We found an overall prevalence of 94% attributed to *Schistosoma mansoni* infection, and a mean of 482 eggs per gram of stool. The preliminary results from this study have revealed an extremely high prevalence of *Schistosoma mansoni* infection in Marolambo, and high parasite loads are indicative of significant disease morbidity. A repeat research expedition in May-June 2016 aims to assess the impact of schistosomiasis on this remote population using anthropometrics, questionnaires, tests for anaemia and assessing cardiovascular fitness.

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EVOLUTION OF IMMUNOLOGICAL MARKERS OF INFECTION WITH *SCHISTOSOMA MANSONI* IN PATIENTS TREATED WITH PRAZIQUANTEL IN THE KOU VALLEY (BURKINA FASO)

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The parasitic diagnosis of infection with *Schistosoma mansoni* using the Kato-Katz technique lacks sensitivity in zones with low prevalence of infection or after chemotherapeutic treatment. The present study aims to evaluate the evolution of immunological markers of *Schistosoma mansoni*'s infection in patients following treatment with praziquantel in the Vallée du Kou. In 2007, 980 subjects having at least 6 years old have been screened at Vallée du Kou (Burkina Faso). Among them, 216 having positif *S. mansoni* stools samples using Kato-Katz test method received a single dose of praziquantel of 40mg/kg, and were followed up from February 2007 to March 2008. During the screening and at each follow-up visits (days 45, 3, 6 and 12 months) stools and blood samples were collected. Subjects found positive were treated with the same single dose of of praziquantel. Kato-Katz test method was used to detect schistosome eggs in stools and the ELISA test was used to detect in the serum antibodies (IgG, IgM, IgA, IgG1, IgG2, IgG3, IgG4), targeting the antigens of schistosome eggs. Results A total of 980 subjects were screened. Their mean age was 23,29±19,42. *Schistosoma mansoni* prevalence in stools samples was 22%. This prevalence significantly decreased overtime (11,4% at 12 month, p=0.001). We observed a regular and significant decrease (p=0.001) of the level of immunoglobulin IgG4. We observed a non-significant reduction in the levels of immunoglobulin IgA et IgG1. There was a positive linear correlation between parasitic load and immunoglobulin IgG4 level before treatment (r=0, 82). During the follow-up this IgG4 immunoglobulin level was significantly lower in subjects having negative Kato-Katz results. In conclusion, the study demonstrated a good efficacy of praziquantel in reducing parasitic infection in treated patients. Immunoglobulin IgG4 is the best marker associated with the therapeutic efficacy of praziquantel in treating *S. mansoni* and could be a possible cure marker.

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RELIABILITY OF COMMUNITY HEALTH WORKER REPORTED TREATMENT COVERAGE FOR SCHISTOSOMIASIS IN WESTERN KENYA-THE SCHISTOSOMIASIS CONSORTIUM FOR OPERATIONAL RESEARCH & EVALUATION PROJECT

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Community health workers (CHWs) are key drivers in mass drug administration programs (MDA). Although the community-directed treatment approach has shown good results in the control of onchocerciasis and lymphatic filariasis,some reports from CHW's treatment coverage have in the past been shown to be inaccurate. We compared CHW reported and household survey coverage for schistosomiasis

to identify factors that may influence CHW reporting. We employed CHWs to conduct community-wide treatment (CWT) in 75 villages in western Kenya. A census was done prior to the MDA to collect demographic data, and all the treatments were recorded in treatment booklets to determine the reported treatment coverage. A household survey was carried out to determine the compliance coverage rates. A structured questionnaire was used to determine treatment coverage levels as well as levels of drug-related side effects.Twenty-four villages were randomly selected for the survey where 1479 households covering 6183 persons were visited in 2012, 2013 and 2014. The eligible population was 5551. Up to 62.9% (53.9-71.9%) reported having been treated compared to CHW's reported coverage of 88.9% (84.2-93.7%) in the same villages (P<0.0047). 33.9% reported being absent during the treatment with 51.3% reporting that the CHW did not visit their homes to offer treatment. More females (50.7%) compared to males (49.2%) complied with treatment. Few people declined treatment for fear of side effects (5.0%). About 2.5% of the population surveyed reported having not heard about the program. Only 1.8% felt they were not sick hence didn't need drugs, while 1.6% reported were pregnant, 1.4% did not take drugs for religious reasons, and 0.05% were influenced by rumors. Of the total population surveyed, only 25.9% experienced side effects with abdominal pains being most frequent (46.0%) followed by diarrhea (31.7%) and dizziness (25.0%).We noted a significant difference between CHW-reported and house-hold survey coverage. The CHW coverage (as validated by the household surveys) improved over time probably attributable to the additional training of the CHWs over the years.

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MODELING THE WITHIN-HOST DYNAMICS OF *SCHISTOSOMA MANSONI*: THE CONSEQUENCES OF INCONSISTENT TREATMENT EFFICACY FOR DISEASE CONTROL

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Schistosomiasis is a neglected parasitic disease caused by various trematode species of the genus *Schistosoma*, for which 249 million people needed treatment in 2012. Substantial variability in treatment efficacy has been observed despite comparable *Schistosoma mansoni* prevalence between populations prior to treatment. Praziquantel is the most common pharmaceutical used to control schistosomiasis, due to its applicability over several species and its negligible side effects. However, Praziquantel is not very effective against juvenile schistosomes in humans. This limited efficacy on the juvenile life-stage of the parasite may be an important factor in the persistence of the disease, yet when forecasting the impacts of control measures this factor is usually neglected. We developed a stochastic model to investigate the consequences of inconsistent drug efficacy among parasite life-stages and variation in parasite population structure within the human host. These results were used to parameterize a population-level model to explore control options, including alternatives to the prevalent annual treatment strategy. The effects of anti-helminths on schistosome population age and sex composition within the human host may obfuscate the effectiveness of chemoprophylactic control strategies. Furthermore, we found the effectiveness of the treatment to be heavily dependent on the force of infection to humans, the initial schistosome population size and structure, and the frequency at which pharmaceuticals are administered. Ultimately our results can be used to design optimal control treatments under differing risk scenarios.

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SYSTEMATIC REVIEW OF ANTISCHISTOSOMAL TREATMENT EFFICACY STUDIES AND THE SIGNIFICANCE OF INDIVIDUAL-LEVEL PARTICIPANT DATA FOR META-ANALYSES

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Schistosomiasis control mainly relies on preventive chemotherapy with praziquantel distributed through mass drug administration. With a target of 260 million treatments a year, reliably monitoring efficacy is all-important. We performed a systematic review of published literature to identify studies that included investigation of antischistosomal drug efficacy, performed since 2000. Out of 914 unique references, we identified 90 studies involving an outcome assessment within 60 days post-treatment, enrolling a total of 20,517 participants infected with *Schistosoma* spp., treated mostly with praziquantel. We extracted study-level characteristics (*Schistosoma* species, treatment, country, methods used for diagnosis and reported estimates of drug efficacy, etc.). This has allowed us to depict the landscape of schistosomiasis research reporting on anthelmintic efficacy assessment, and to highlight the associated diversity in methodological and reporting approaches. We complete this descriptive exercise by meta-analyses, exploring spatial and temporal heterogeneity among estimates of antischistosomal drug efficacy. We will discuss our results in the context of global efforts to control schistosomiasis. Our work highlights the general limitations of aggregate-data meta-analyses. We argue that individual participant-level data (IPD) would allow application of standardised analytical approaches to efficacy and safety assessments, and more powerful investigation of treatment effects in target sub-populations (as demonstrated by our prior IPD meta-analyses on a subset of up to 4,740 participants). This leads us to discuss the importance of initiating and sustaining efforts to collect and share clinical and epidemiological data, and the potential benefits of this approach to the effective monitoring of antischistosomal drug efficacy.

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OUTBREAK OF GASTROENTERITIS ASSOCIATED WITH BORE WELL WATER - GANESHPUR VILLAGE, BIDAR DISTRICT, KARNATAKA STATE, INDIA, 2015

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Diarrhea and foodborne outbreaks account for half of outbreaks in India but few investigations identify the source. On October 9, 2015, Bidar district of Karnataka state, India, reported a gastroenteritis outbreak in Ganeshpur village. We investigated to identify risk factors and provide recommendations. We defined a case as ≥3 loose stools within 24 hours between September 3 and November 3, 2015 in a Ganeshpur village resident. We conducted a retrospective cohort study to assess risk factors and collected stools for Vibrio cholerae culture. We collected one water sample from bore well A and six water samples from bore well B to

test for faecal contamination. We interviewed 947/1040 villagers (91% response rate) and identified 180 cases (attack rate=19%) and 2 deaths. Among 180 cases, 90% were admitted. The outbreak occurred between September 5 and November 1 and peaked October 5-8. Having family members with diarrhoea (RR=4.9, 95% CI=3.5 – 7.1) and using water from bore well A for drinking or cooking (RR=2.6, 95% CI=2.0 – 3.4) were associated with illness. Using water from bore well B for drinking or cooking (RR=0.4, 95% CI=0.3-0.5), drinking filtered water (RR=0.4, 95% CI=0.3 – 0.6), washing hands before eating (RR=0.6, 95% CI=0.4 – 0.7) and having a toilet at home (RR=0.2, 95% CI=0.1 – 0.6) were protective against illness. Stool cultures from four case-patients were negative for Vibrio cholerae. Water from bore well A was positive for faecal contamination by most probable number test. All other water sources were potable. This gastroenteritis outbreak was likely due to consuming contaminated water from bore well A. We recommend avoiding bore well A, using bore well B water, promoting hand washing practices, and improving access to indoor toilets.

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QUANTITATIVE ASSESSMENT OF EXPOSURE TO FECAL CONTAMINATION FOR YOUNG CHILDREN IN A CROWDED, LOW-INCOME URBAN ENVIRONMENT IN THE SANIPATH STUDY OF ACCRA, GHANA

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Diarrheal diseases are a leading cause of death for children under five globally. In the developing world, lack of adequate sanitation results in fecal contamination of the environment and poses a risk of enteric disease transmission via multiple exposure pathways. To better understand how different sources and transmission routes contribute to overall exposure to fecal contamination, we identified eight different fecal exposure pathways for children under five years old in four high-density, low-income neighborhoods in Accra, Ghana, and quantified the contribution of each pathway to oral intake of fecal contamination. Data collection for the SaniPath study was from 2011 to 2012, and comprised 500 hours of structured observations for behaviors of 156 children, questionnaires from 800 households, and 1855 environmental samples for microbiological testing. Data were analyzed using Bayesian models, estimating the environmental and behavioral factors associated with exposure to fecal contamination. These estimates were applied in exposure models simulating sequences of behaviors and transfers of fecal indicators from the environment to oral ingestion. This approach allows us to identify the contribution of any sources of fecal contamination in the environment to child exposure and use dynamic fecal microbe transfer networks to track fecal bacteria from the environment to oral ingestion. Exposure pathways were categorized into four types (high/low by dose and frequency), as a basis for prioritizing pathways by their potential to reduce fecal exposure. Although we observed variation in exposure (magnitude ranged from 108 to 1016 CFU/day for E. coli) between different age groups and neighborhoods, the greatest contribution consistently was through the food pathway (contributing >99.9% to total exposure) in Accra, Ghana. Hands played a pivotal role in fecal microbe transfer from the environment to ingestion. The fecal microbe transfer network provides a systematic approach to study the complex interaction of poor sanitation infrastructure and human behavior on exposure to fecal contamination.

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OBSERVATIONAL AND LABORATORY HYGIENIC ANALYSIS OF RESTAURANTS IN IQUITOS, PERU

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Poor sanitation contributes to foodborne infections associated with food consumption. Specific risk factors associated with contamination include unsafe handling and preparation of food, hygiene, sanitation, and water quality. Fifteen restaurant owners in Iquitos, Peru participated in a study to assess sanitation and hygienic practices. The study included an observational survey and laboratory analysis of samples collected from food, water, and the restaurant environment. Participants were given a final report of the findings. Survey questions included food handling and preparation, restaurant cleanliness, sanitation, and standardized hygienic practices. Observations were numerically graded and an average sanitation score was calculated. Restaurant contact surfaces, food, and beverages were cultured for enteric bacterial pathogens via sample collection onto rayon or polyester swabs in Stuart's transport media with same day culture according to standard microbiology protocols. Water samples were collected for fecal coliform testing. Statistical significance was determined using a chi-square analysis. There was no significant correlation between average sanitation scores and the presence of enteric pathogens found on contact surfaces or food samples. The sanitation scores for the 15 restaurants ranged from 26 to 43.38. Presence or absence of enteric bacteria was assessed and results ranged from none isolated to one or more present. Coliform counts ranged from to <1 to 21 UFC/100mL. The restaurant with the lowest sanitation score average (26) yielded no growth of enteric pathogens and no fecal coliforms. Conversely, the restaurant with the highest sanitation score average (43.38) yielded enteric pathogens but no fecal coliforms. Overall, no statistical significance was noted between bacterial growth and the presence of fecal coliforms in the cooking water. Additionally, there does not appear to be a correlation between In observational analysis and results of coliform analysis or enteric bacterial culture. Future studies may include a resurvey of restaurants to assess reproducibility and potential interventions.

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LATRINE LEARNING: USING CONDITIONAL INFERENCE TREES TO EXPLORE HOW LATRINE CONDITIONS CAN PREDICT LATRINE USE IN RURAL BANGLADESH

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Global public health efforts to eliminate open defecation specifically on the Indian subcontinent have recently begun focusing on improving latrine use. In this study, we attempt to identify a latrine's likelihood of use based on observations of physical characteristics of the latrine and the surrounding premises (i.e., latrine spot-check indicators [SCIs]). Recursive partitioning algorithms, often called decision trees, are typically used in machine learning and data mining because they do not require the assumptions made by traditional regression models. Conditional inference trees (CIT) specifically apply unbiased statistical inference tests as a method of variable selection based on a priori partitioning criteria. Unlike other regression trees, the selected partitions are conditional of all other covariates in the model. In this study we measured latrine usage in rural Bangladesh in 2014 using average daily 'likely defecation events' recorded by a motion sensing device called a passive latrine use monitor (PLUM). Using this continuous distribution, we dichotomized the

measurement along its median so that we had a "Most used" group (≥ median) and a "least used" group (< median). We then employed CIT to separately predict the continuous and dichotomous forms of the outcome using 15 SCIs as independent variables. After implementing a Bonferroni correction for multiple tests of significance, the CIT analysis identified a tree with three partitions using three SCIs for the dichotomous outcome. The primary partition was the presence/absence of water for the purpose of flushing or anal cleansing with two secondary partitions being 1) the presence/absence of flies and 2) having a wet floor. The primary partition shows the strongest SCI but the secondary partitions show that a latrine with water for cleansing that does not attract flies and latrines that do not have water for this purpose but keep a dry floor draw the most use from their users. This interaction suggests a latrine's cleanliness and structural maintenance is an important indication of its use. The CIT for the continuous outcome could indicate some measurement error within the PLUMs.

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WOMEN AND WATER USE IN THE EASTERN REGION OF GHANA: A QUALITATIVE APPROACH

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Despite efforts to expand access to improved water sources in sub-Saharan Africa, countries like Ghana continue to face significant heterogeneities in water source coverage and quality. When multiple water sources are available for use, it is critical to understand the decision-making process in water source selection. Given the key role played by women in Ghana with respect to household water resources management, we interviewed 50 women from 11 communities in the Eastern Region of Ghana and asked about preferences for improved and unimproved water source use. Data was used to compare water source coverage with actual water use and women's water source preferences; questions relate to perceptions of "good" and "bad" water, availability and accessibility, and seasonality of water sources. Concepts such as "improved water" do not always match the definitions women have of "good" water. Women assess water appropriateness for various domestic tasks using a complex set of factors, such as contextual indicators, social conceptualizations of "good" and "bad" water, organoleptic assessments, and seasonally-determined quality and availability. Distance to the water source plays a key role in source acceptability. While rainwater is overwhelmingly preferred for domestic use and is used by all participants during the rainy season, source preferences change in the dry season. Women can articulate their water source preferences, but many find it challenging to consistently access a water source that provides "good" quality water. This is due to factors like distance, unmaintained or unavailable infrastructure, and visible and/or biochemical contamination. Water source availability is partially determined by the season and impacts women's water source use year-round. Our research contributes to a qualitative, community-based approach to assessing the need for improvements in water quality and accessibility in a schistosomiasis-endemic part of Ghana.

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IDENTIFICATION OF CAUSAL PATHWAYS DETERMINING THE RELATIONSHIP BETWEEN PATHOGEN-SPECIFIC INFECTION AND IMPAIRED GROWTH AMONG CHILDREN < 59 MONTHS IN MIRZAPUR, BANGLADESH

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The burden of childhood diarrhea and malnutrition remains high in South Asia due to inadequate household sanitation, lack of access to improved water and poor hygiene practices. We evaluated causal pathways linking household factors, enteric pathogen infections (EPI) and impaired growth using data from Mirzapur, Bangladesh that is part of the Global Enteric Multicenter Study. Stool specimens collected at enrollment from children with moderate-to-severe diarrhea and matched controls were screened for bacterial, viral and protozoa EPI. Height measurements of children were taken of children at enrollment and information was collected on sanitation facilities, water sources, and household animals, cooking fuel type, caretaker education and hand washing practices. Structural equation models tested pathways directly linking household factors with stunting (< - 2 height-age-Z score) or indirectly through their effects on EPI transmission. The modifying effects of hand washing behaviors, water sources and caretaker education were also tested. *Giardia lamblia* and *Cryptosporidium* infections were associated with increased stunting among older children. In turn, a higher prevalence of *Cryptosporidium* was associated with cow dung fuel use when caretakers reported no hand washing before eating. Traditional latrine use was associated with a greater prevalence of *G. lamblia* and *Cryptosporidium* infections when caretakers reporting no hand washing before cooking. A higher prevalence of *Cryptosporidium* was also associated with child feces disposal when caretaker had no formal education. Increased caretaker education had the greatest effect on stunting through direct associations with reduced stunting and indirectly through effects on *Cryptosporidium* and *Giardia* infections. Overall, causal pathways were identified that linked animal and environmental reservoirs with childhood stunting that were modified by distinct hygiene-related behaviors. These results can be used as tools to inform the design, implementation and evaluation of different interventions to more effectively reduce diarrhea burden and stunting.

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HIGH PREVALENCE OF *BLASTOCYTIS* SP IN POPULATION LIVING IN URBAN AREAS OF GABON

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Intestinal parasitic infections (IPIs) are one of the major public health problems, especially in the rural area of developing countries with low socio-economic status and poor sanitation. The aim of the study was to establish the profile of intestinal parasites diagnosed at the Department of Parasitology-Mycology (DPM) of the Faculty of Medicine in Gabon over a period of 10 years. A retrospective study was carried out. Socio demographic and clinical/biological data of patients, who consulted at the DPM between 2004 and 2014 and who benefited of stool examination, have been collected from archived files. During the survey, 1251 patients have been selected; 81% of them were adults. Half of them lived in urban areas, a quarter (25.1%; n=118) in slums and 20.4% (n=96) in rural

and semi-rural areas. IPIs have been found in 39.8% (497/1251) of the patients, mostly Protozoan: 84.9% (n=422/497) vs 15.1% (n=78/497) of helminths. Overall, 13 parasites species have been identified, the predominant was sp (45.1%; n=224/497) followed by *Entamoeba (E.) coli* (42.5%; n=211/497). *Blastocystis* sp (41.6% (n=42/101), *E. coli* 32.7% (n=33/101) and *E. nanus* 27.7% (n=28/101) were more frequently detected among populations living in slums (p=<0.0001). Prevalence of intestinal parasites increased between 2004 and 2014 (p<0.01) ranging from 34.1% to 65.3% between 2011 and 2014; due to the elevated frequency of Protozoan. None *Blastocystis* sp was found in samples collected in 2004, but its prevalence rose significantly from 2010 reaching 23.7%, in 2013 and 22.3% in 2014. Soil Transmitted Helminths prevalence varied during the study period. A decrease of the prevalence was observed being below 10%. An increase of the Protozoan frequency was found among patients living in urban areas and slum compared to populations living in rural areas (p<0.01). These data showed a non negligible rise of frequency of *Blastocystis* sp infections in populations living in urban areas of Gabon. Integrated efforts, such as improving infrastructure to provide clean water source and educating the inhabitants for appropriate hygienic lifestyle are needed.

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FORMATIVE RESEARCH TO INFORM DESIGN OF A BEHAVIOR CHANGE INTERVENTION FOR THE "F" AND "E" COMPONENTS OF THE SAFE STRATEGY FOR TRACHOMA CONTROL IN OROMIA, ETHIOPIA

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Trachoma, caused by *Chlamydia trachomatis,* is thought to be transmitted from eye to eye via hands, fomites and eye-seeking flies which breed in human faeces. Little research has been conducted to understand hygiene and sanitation-related behavioural risk factors for trachoma transmission or how these might be addressed through the "F" and "E" (face washing and environmental change) components of the SAFE strategy. This impedes progress towards the 2020 trachoma elimination goal. A study was carried out in a trachoma hyper-endemic area in Oromia, Ethiopia to identify behaviours potentially associated with trachoma risk, namely: water use for hygiene purposes; defecation and stool disposal practices; sleeping arrangements and laundry. Data were collected in five communities in January 2016 through direct observation in households with young children (n=10), semi-structured interviews with caregivers (n=10), focus groups with mothers (n=3), grandmothers (n=1) and fathers (n=1) and stakeholder interviews (n=4). Data collection and analysis were guided by an ecological model of the determinants of behaviour: "Evo-Eco". A range of sub-optimal hygiene practices were documented, but none were consistently poor across households. Open defecation within or next to a compound was a normative practice, particularly for young children. Latrines, when present, had been built under threat of a fine, and were unhygienic and poorly constructed. Faces were washed with hands and feet, occasionally in response to a cue (food or dirt) or for refreshment. Frequency of face washing differed within and between households and soap was intermittently used. Children's dirty faces with visible discharge were not viewed to be disgusting and were rarely wiped by mothers who felt the ideal behaviour was unattainable for them. Families slept closely together and shared bedding. Laundry was done infrequently. Laundry and bedding were not thought to transmit germs. Behaviour change activities should focus on making face washing feasible and a priority for all family members, generating demand for sanitation and promoting use and laundry of affordable pillows.

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INCORPORATING WASH INDICATORS INTO NATIONAL CONTROL PROGRAM SURVEYS FOR SCHISTOSOMIASIS AND STH IN MADAGASCAR

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Access to clean water, sanitation and hygiene (WASH) has been recognised as a key element of socio-economic development, and has been adopted as one of the Sustainable Development Goals. Recent studies show interesting links between access to WASH and the reduction of transmission of schistosomiasis and soil-transmitted helminthiasis (STH); however, reports also highlight the need for more data collection to better understand the factors involved. In October 2015, a baseline study was conducted in the western half of Madagascar prior to scale-up of the schistosomiasis and STH control programme. Primary outcomes were to determine the prevalence and intensity of schistosomiasis and STH in the treatment area. Indicators of access to, and use of, WASH facilities as defined through consensus-defined observed and reported factors were included as secondary outcomes in order to determine the feasibility of incorporating collection of school-level data on access to WASH, and to determine the relationship between access to school-level WASH and schistosomiasis and STH. A total of 1,958 children were tested from 29 primary schools across the treatment area. The prevalence of *S. haematobium* infection and heavy-intensity infection was 30.5 % and 15.1 %, respectively. The prevalence of *S. mansoni* infection and heavy-intensity infection was 5.0 % and 0.9 %, respectively. The prevalence of any STH was 4.7%. Of a total WASH score of 12, 75% of schools scored less than 3, and the maximum score was 7. This study demonstrates the feasibility of including WASH indicators in monitoring and evaluation surveys for neglected tropical disease control programmes. The results show an extremely limited access to WASH in the study sites. No significant relationships were observed between WASH score and school level infection rates, perhaps due to sample size limitations. The national control programme will aim to integrate interventions to improve access to WASH alongside mass treatment of infection.

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ESTIMATING THE GLOBAL RISK OF DIARRHEAL DISEASE ATTRIBUTABLE TO INTERMITTENT WATER SUPPLIES

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Approximately 925 million people globally are served by an intermittent water supply (IWS). These supplies are often fecally contaminated and are associated with increased risks of gastrointestinal illness. The global burden of disease associated with such supplies however is unknown. A quantitative microbial risk assessment (QMRA) was performed using Monte Carlo techniques to estimate the risk of infection for three reference pathogens (*Campylobacter*, *Cryptosporidium*, rotavirus) attributable to consuming contaminated tap water supplied by an IWS. The stochastic model utilized *E. coli* measurements in intermittent distribution systems along with reference pathogen to indicator bacteria ratios in three potential sources of contamination (sewage, surface water, groundwater) to characterize risks of infection. These risks of infection were then used to calculate the annual diarrheal cases, DALYs, and deaths that might plausibly be associated with IWS. Results indicate that when considering contamination of an IWS by sewage, the daily risks of infection are 1 in 480 for *Campylobacter*, 1 in 885,000 for *Cryptosporidium*, and 1

in 61,000 for rotavirus. Collectively, these reference pathogens and their median daily risks of infection are estimated to lead to approximately 143.5 million cases of diarrhea annually and cause 1.98 million associated DALYs and 31,250 deaths. Calculated DALYs and deaths associated with IWS in this analysis account for 6.6% and 7% respectively of the total diarrheal DALYs and deaths attributable to inadequate water according to recent burden of disease estimates. This is the first attempt to estimate the risk of specific enteric infections, and global diarrheal disease burden expressed in cases, DALYs and deaths, associated with intermittent water supplies using QMRA. These results suggest that there may be significant health risks associated with intermittency of water supplies to which almost 1 billion people are exposed.

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IMPACT OF COMMUNITY HEALTH CLUBS ON DIARRHEA, ANTHROPOMETRY AND WATER QUALITY IN WESTERN RWANDA

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The Community-Based Environmental Health Promotion Programme (CBEHPP) is a cluster-randomized controlled trial that covers 150 villages in Rusizi district, western Rwanda. The intervention was community health club meetings led by trained facilitators. Villages were randomly assigned to one of 3 study arms: control (no intervention), Lite (8 meetings), or Classic (20 meetings). We aimed to evaluate the impact of the community health club approach on diarrhea among children <5, anthropometry among children <2, and household water quality. The CBEHPP endline survey collected data in late 2015 on diarrhea in the previous 7 days for children <5 (N=10,261), anthropometry for children <2 (N=3,179), and water quality for a random sub-sample of households (N=1,085). To analyze impact on diarrhea, we used log-binomial regression with a log link function and generalized estimating equations (GEE). For anthropometry, we calculated length-for-age and weight-for-length z-scores (LAZ and WLZ) then used linear regression with GEE. For water quality, we measured colony forming units of thermotolerant (fecal) coliforms (TTC) per 100ml water then used linear regression with GEE. All analyses accounted for clustering at the village level and the household level as appropriate. The prevalence of caregiver-reported diarrhea for children <5 in the previous 7 days was 14.2%, 14.2%, and 14.3% in the control, Lite, and Classic arms respectively. Mean (SD) LAZ among children <2 was -1.54 (1.26), -1.59 (1.28), and -1.61 (1.32) in control, Lite, and Classic respectively. Mean (SD) WLZ among children <2 was 0.18 (1.11), 0.18 (1.09), and 0.10 (1.11) in control, Lite, and Classic respectively. Mean (SD) TTC was 139.8 (230.9), 165.4 (251.1), and 155.5 (243.7) in control, Lite, and Classic respectively. The differences between study arms were not statistically significant for any outcomes (p=0.96, 0.20, 0.83, 0.47 for diarrhea, LAZ, WLZ, and TTC respectively). The community health club model, as implemented in this setting in western Rwanda, had no impact on prevalence of diarrhea or anthropometry among children or on household water quality.

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THE ROLE OF PUBLIC-PRIVATE PARTNERSHIPS FOR NEGLECTED TROPICAL DISEASES (NTDS) PREVENTION AND CONTROL: THE SUPER SCHOOL OF FIVE TRACHOMA PROGRAM

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Public Private Partnership (PPP) is an increasingly popular model for implementing important public health intiatives. Sighstavers international through its partnership with Unilever launched a school-based trachoma program that integrates face washing into the existing Unilever's Lifebuoy brand school of five handwashing programs, towards the prevention and control of trachoma. This program is delivered in collaboration with local ministries of health and education in-country. The super-school of five (SS5) program seeks to increase hygiene behaviors and in particular the practice of hand and face washing with soap among school children and their mothers/caregivers. Specifically, it addresses the facial cleanliness component of the SAFE strategy for trachoma control. This program adopts the Unilever behavioral change model and takes school children through a four-step journey of awareness, commitment, reinforcement and reward/recognition. Each step on the behavioral change pathway has a corresponding communication material. The SS5 program has been rolled out in 41 primary schools in the trachoma endemic county of Turkana in Kenya. Currently, the SS5 is being scaled-up across trachoma endemic countries in the region. Pre-and post-evaluation comparisons following the roll out of the SS5 showed marked improvements in facial washing and handwashing events (21.2% vs. 75.6%) and face and handwashing events using soap (0 vs. 75.4%). Public private partnerships have the potential for meaningful benefits to be gained for the public partner and the overall health sector. The super school of five program demonstrates that partnerships with the private sector can also be particularly valuable as a method of leveraging on existing models and technical expertise all of which can lead to the ultimate goal of trachoma elimination. The super school of five model and adapted materials along with results from the current ongoing programs is briefly presented.

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RELATIONSHIP OF INFANT DIET TO CHILDHOOD HEALTH: ROUTES OF PARASITIC INFECTIONS FROM CONTAMINATED WEANING FOODS

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There is a deficit of published data on the prevalence and intensity of gastrointestinal parasitic infections in Honduras and routes of parasitic transmission especially in infants and young children. Historically, many of the surveys of water-borne, vector borne and soil-transmitted parasites were published more than 30 years ago. Transmission of parasitic infections in Honduras are the result of many factors which include zoonotic transmission, contaminated water and foods, direct transmission from child care providers, food handlers, and agricultural workers; each playing a contributing role in the transmission of parasitic diseases. From November 2014 through February 2015, the Department of Microbiology, VCOM, conducted a preliminary 3 month-long study in Honduras where 175 mothers were extensively interviewed. This study investigated weaning practices used by mothers when transitioning infants from breast milk to complementary foods and the role these foods have in the transmission of gastrointestinal parasites. Of those surveyed, 98.9% of mothers reported at least one of their children infected with a gastrointestinal parasite. Out of the 322 children of the mothers surveyed, 42% of the children had been previously diagnosed with a gastrointestinal parasitic infection. In

this study routes of infection of protozoan and helminth parasites could have resulted from contaminated complementary foods and water given to infants while still breastfeeding or from contaminated foods after breastfeeding was completed. Contaminated water is a likely source of protozoan parasites. Contaminated water was fed to infants directly, used to mix with formula or complementary foods, or to wash bottles for infant feeding. There was an absence of hand washing by children and mothers before eating or while preparing foods. The major source of soil transmitted helminth infections was the result of environmental contamination, unwashed or uncooked complementary foods, unpasteurized animal milk, unsanitary food storage, poor living conditions with exposed dirt floors, and exposure to roaming domestic animals.

1201

DETERMINANTS OF LIFE-THREATENING DIARRHEAL DISEASE AT HOSPITAL PRESENTATION: EVIDENCE FROM 22 YEARS OF ADMISSIONS IN BANGLADESH

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To take advantage of emerging opportunities to reduce morbidity and mortality from diarrhoeal disease, we need to better understand the determinants for life-threatening disease in resource-poor settings. We analyzed records of patients admitted with acute diarrhoeal disease over twenty-two years at the International Centre for Diarrhoeal Disease Research, Bangladesh (1993-2014). Patients presenting with and without severe dehydration (SD) were compared by multivariable logistic regression models, which included socio-demographic factors and pathogens isolated. Generalized additive models evaluated non-linearities between age or household income and SD. Among 55,956 admitted patients, 13,457 (24%) presented with SD. *Vibrio cholerae* was the most common pathogen isolated (12,405 patients; 22%), and had the strongest association with SD (AOR 5.88; 95% CI: 5.52-6.27); detection of multiple pathogens did not exacerbate SD risk. The highest proportion of severely dehydrated patients presented in a narrow window only 4-12 hours after symptom onset. Patients between 10-15 years had the highest probability of presenting with SD, with dramatic increase per year of life up to age 10. Adult women had a 38% increased odds (AOR 1.38; 95% CI: 1.30-1.46) of SD compared to adult men. The probability of SD increased sharply at low incomes. These findings were consistent across pathogens. There remain underappreciated populations vulnerable to life-threatening diarrhoeal disease that include children 10-15 years-old and adult women. In addition to efforts for children under 5 years, there is an urgent need to develop interventions for these older sub-populations that are accessible within 4 hours of symptom onset.

1202

BISMUTH SUBSALICYLATE REDUCES ANTIMICROBIAL USE AMONG ADULT DIARRHEA PATIENTS IN PAKISTAN: A RANDOMIZED, PLACEBO-CONTROLLED, TRIPLE-MASKED CLINICAL TRIAL

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The World Health Organization has declared antimicrobial resistance a global crisis. Antimicrobial medications are used inappropriately for many of the 2 billion annual cases of diarrhea. We assessed whether providing bismuth subsalicylate (BSS) to adults with acute diarrhea could reduce antimicrobial use in a setting where antimicrobials are easily obtained. We included adult outpatients from 22 clinics in Karachi, Pakistan, who presented from Apr - Oct 2014 with acute diarrhea and who had not taken antimicrobial or antidiarrheal medications during the present illness. Twenty patients from each clinic were randomized 1:1 to BSS or placebo. We requested stool specimens for bacterial culture upon enrollment and assessed antimicrobial use during 5 days of follow-up. We present unadjusted odds of antimicrobial use by intervention because we did not detect interactions with clinic, age, sex, wealth, or diarrhea severity. We concealed group assignment from patients, healthcare providers (HCPs), field staff, and the principal statistician. Among 440 participants, those who received BSS were less likely than those who received placebo to use antimicrobials (odds ratio [OR] 0.55, 95% confidence interval [CI] 0.30 - 0.98), particularly when ≥1 pathogen was detected (OR 0.26, 95% CI 0.09 - 0.81). Fifty-four (12%; BSS, 20; placebo, 34) participants took antimicrobial medications; all received them from a HCP, 36 (67%) began taking them within 1 day of enrollment, 25 (46%) were treated with >1 antimicrobial agent (mean 1.6 ± 0.7 oral and 0.6 ± 0.8 intravenous agent), and nitrofurantoin (n=46 [85%]) and ciprofloxacin (n=28 [52%]) were the most commonly used agents. Six (1%) patients were hospitalized (BSS, 3; placebo, 3); no patients died. Providing BSS to adults with acute diarrhea reduced antimicrobial use by 45% in a setting with high rates of diarrhea and antimicrobial usage for diarrhea. Encouraging HCPs and pharmacists in similar settings to recommend BSS as front-line treatment for adults with diarrhea, and promoting BSS for diarrhea self-management, may reduce antimicrobial use and rates of antimicrobial resistance globally.

1203

CONTINUED FEEDING DURING DIARRHEA MANAGEMENT AT HOME AND GROWTH FALTERING: SECONDARY DATA ANALYSIS OF THE KENYA GLOBAL ENTERIC MULTICENTER STUDY (GEMS) OF DIARRHEAL DISEASE IN INFANTS AND YOUNG CHILDREN IN DEVELOPING COUNTRIES

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In 2013, 587,000 child deaths were attributed to diarrhea. WHO guidelines recommend commercially available oral rehydration salts (ORS), zinc, and continued feeding of an age-appropriate diet for diarrhea. From 2006-2011, 34% of children in sub-Saharan Africa received continued feeding and ORT during diarrhea. Among case data from the Global Enteric Multicenter Study (GEMS) Kenya site, we investigated the association between continued feeding at home during a moderate-to-severe diarrhea episode (MSD) in children< 5 years old (reported at enrollment by a caregiver) and growth faltering 50-90 days after enrollment. At enrollment, caregivers were asked about feeding practices at home during the MSD episode, with continued feeding defined as offering a usual or more than usual amount of food during MSD. Weight and height were measured at enrollment and at follow-up 50-90 days later. Growth faltering was defined as a *reduction* in weight-for-height Z-score (WHZ) category by >1 SD from enrollment to follow-up. We evaluated the association between continued feeding during MSD and growth faltering using log binomial regression analysis. Data from 1,363 of 1,476 enrolled children with MSD (92%) was complete; 20% received continued feeding and 26% experienced growth faltering. The mean age of children provided continued feeding was higher (20 months, SD 13) than of those who had feeding discontinued (17 months, SD 13). Vomiting 3 or more times a day was inversely associated with continued feeding (RR=0.69, 95% CI=0.58, 0.82). Caregiver's wealth and education were not associated with continued feeding. In bivariate analysis, children

reported to have received continued feeding during MSD had 25% lower risk of growth faltering (267, 20%), compared to those who had feeding discontinued (1096, 80%) (RR=0.75, 95% CI=0.59, 0.97); the association was confounded by age (RR=0.86, 95% CI=0.67 - 1.09). Kenyan children with MSD were rarely provided continued feeding but age-adjusted analyses suggested that continued feeding did not significantly alter the risk of growth faltering. Barriers to feeding during diarrhea include young age and frequent vomiting.

1204

DISRUPTIONS OF THE HUMAN GUT MICROBIOME DURING DIARRHEA INFECTIONS CAUSED BY ROTAVIRUS AND ENTERO-PATHOTYPES OF *ESCHERICHIA COLI*

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Little is known about the disturbances that enteropathogens cause in the ecology of the normal gut during diarrheal infections. For example, it is unclear whether viral and bacterial pathogens produce similar or distinctive alterations in the structure of the human gut microbiome and it remains undetermined whether pathogen-specific signatures in the sick gut microbiome exist that discriminate among different pathotypes. In this study we report on an analysis of 74 cases of human diarrhea in Northern Ecuador where six different pathotypes of *Escherichia coli* were identified as the probable causative agent and compare these to 11 cases of diarrhea where rotavirus was detected. We compared these samples to those from 54 healthy samples and 13 samples from other cases of diarrheal disease of undefined etiology, from individuals living in the same geographical locations. We used 16S rRNA taxonomic profiling combined with metagenomic sequencing to assess OTU richness, evenness and diversity in both diarrhea and healthy samples and evaluated the effects of additional demographic variables in the variation observed at the taxonomic and functional level. Our preliminary results have shown a significant reduction in OTU richness and diversity in individuals with diarrhea of undefined etiology when compared with healthy samples (paired *t*-test, $p = 0.01$) and that when the causative agent was specified, such as in rotavirus and *E. coli* infections, the diarrheal samples were distinguishable from healthy ones based on distinct taxonomic and metabolic differences. We have also observed that geographical location and age (but not sex or ethnicity) are important factors determining the structure of the healthy gut microbiome in individuals from Northern Ecuador and play a significant role in the response of the gut microbiota to perturbations generated during diarrheal disease. Together, our results advance our understanding of how the ecology of the healthy gut microbiota is disrupted during pathogen-specific diarrheal infections and provides insight into the role of demographic factors in determining gut microbiome response to enteric infections.

1205

THE FECAL MICROBIOME ASSOCIATED WITH SMALL INTESTINE BACTERIAL OVERGROWTH IN BANGLADESHI CHILDREN

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Small intestine bacterial overgrowth (SIBO) has been associated with intestinal inflammation, linear growth delay, and poor sanitation in children from low-income countries. Neither the microbiota that comprises SIBO in this setting nor association with enteric pathogens has been reported. We tested for SIBO via glucose hydrogen breath testing at 2 years of age in a cohort of 90 Bangladeshi infants. These children had their stool microbiome assessed via 16s rDNA sequencing and analyzed via linear discriminant analysis (LDA) effect size (LEfSe) to determine the influence of the fecal microbiome on presence of SIBO. Additionally, multiplex PCR for 30 enteric pathogens was conducted utilizing a Taqman array and analyzed via Fisher's Exact Test. 15 (16.6%) of the 90 children tested were SIBO positive. Children with SIBO had a statistically significant increase in Lactobacillus spp. (LDA score 3.46, p value 0.03) and a decrease in Megasphaera spp. (LDA score 2.38, p value 0.04). There was no difference in Shannon Diversity indices between SIBO positive and negative children. Pathogen carriage did not differ between the two groups for any of the pathogens studied. However, SIBO positive children carried more pathogens on average in their stool than the SIBO negative group (4.2 vs 3.75 pathogens, p value 0.037). Although changes in the stool microbiome were noted, the duodenal microbiome needs further investigation. No association with a particular pathogen was found and increased asymptomatic pathogen burden is likely a marker of poor sanitation, which is known to predispose to SIBO.

1206

EVALUATION OF THE TEST-NEGATIVE CASE-CONTROL DESIGN TO ESTIMATE ROTAVIRUS VACCINE EFFECTIVENESS IN LOW-INCOME SETTINGS

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The test-negative case-control study design (TND) is an epidemiologic method currently used to measure rotavirus vaccine (RV) effectiveness. In this design, vaccination status is compared between rotavirus-positive cases and rotavirus-negative controls presenting to the clinic and meeting a pre-defined case definition for acute gastroenteritis. Despite the use of this study design in low-income settings, the TND has not been validated to measure rotavirus vaccine effectiveness. This study builds upon prior methods to evaluate the TND for influenza vaccine by using a randomized controlled clinical trial database. Similarly, test-negative vaccine effectiveness (VE-TND) estimates were derived from three large

randomized placebo-controlled trials (RCTs) of monovalent (RV1) and pentavalent (RV5) rotavirus vaccines in sub-Saharan Africa and Asia. Derived VE-TND estimates were compared to the original RCT vaccine efficacies (VE-RCTs). VE-TND and VE-RCT estimates were almost identical during the first year of life (RV1: VE-TND (95% Confidence Interval): 58.2% (35.5-72.9), VE-RCT: 61.2% (44.0-73.2); RV5- sub-Saharan Africa: VE-TND: 66.9% (42.7-80.9), VE-RCT: 64.2% (40.2-79.4); RV5- Asia: VE-TND: 44.4% (-13.2-72.2), VE-RCT: 51.0% (12.8-73.3)). Point estimates were also comparable using additional diarrheal surveillance into the second year of life, though the VE-TND overestimated the VE-RCT in the RV5 trial in sub-Saharan Africa. Analyses restricted to the second year of life replicated the limited vaccine efficacy demonstrated in the RCTs. In conclusion, TND vaccine effectiveness estimates were nearly equivalent to original RCT vaccine efficacies, with some country-specific differences. In the RV1 and RV5 trials there were challenges in case capture and varied methods of surveillance between study sites. Limitations of the original RCTs should be considered when comparing derived effectiveness and primary efficacy results. Nevertheless, this study supports the feasible and efficient TND as a valid study design to measure rotavirus vaccine effectiveness in low-income settings.

1207

A PHASE 1 OPEN-LABEL, DOSE ESCALATING STUDY OF ARTIFICIAL *SHIGELLA FLEXNERI* 2A INVAPLEX ADMINISTERED INTRANASALLY TO HEALTHY, ADULT VOLUNTEERS

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An efficacious *Shigella* vaccine is a coveted public health intervention. The intranasal (IN) route of mucosal delivery has shown promise for subunit vaccine administration. The *Shigella* artificial invasin complex (termed, InvaplexAR) contains IpaB, IpaC and *S. flexneri* 2a LPS. The primary objective of this study was to evaluate the safety of InvaplexAR given by IN immunization (without adjuvant) assessed by active monitoring during the vaccination stage and for 28 days following the third vaccine dose. The secondary objectives were to evaluate immune responses and thus identify a safe and immunogenic dose of InvaplexAR for advancement to preliminary efficacy studies or expanded Phase 1 trials. The study is an open-label, dose-escalating first-in-human trial in which volunteers received one of four vaccine doses (10, 50, 250 or 500 µg). We have immunized all four cohorts enrolling 37 subjects with safety data available for the first three cohorts (27 volunteers). The vaccine was administered on Days 0, 14, and 28 in a total volume of 200 µL split equally between both nostrils and delivered with a nasal spray device (VaxINator ™ distributed by Teleflex). Blood, stool, saliva and ocular secretions were collected at specified intervals to examine systemic and mucosal immune responses directed to Invaplex, LPS, IpaB and IpaC. Peripheral blood mononuclear cells were also collected to determine IgA antibody secreting cells and antibody lymphocyte supernatant responses. IN immunization with InvaplexAR was well tolerated. The overall safety and tolerability profiles were consistent with prior IN immunizations. There were no adverse events (AEs) that met the vaccination stopping criteria. Most vaccine-related AEs were of mild severity. Only three subjects reported AEs of moderate severity. The most commonly observed AEs were rhinorrhea (0-33%), nasal congestion (11-33%), nasal tenderness, itching and burning (0-33%). The VaxINator ™ consistently delivered 200 µl ± 10% of InvaplexAR. Immunological assessments are currently underway and will help inform future clinical evaluations of the subunit *Shigella* vaccine.

1208

ASSESSMENT OF THE NEEDS FOR THE NEGLECTED TROPICAL DISEASE (NTD), NON-COMMUNICABLE DISEASE (NCD) AND THE EYE HEALTH PROGRAMS IN LIBERIA FOLLOWING THE OUTBREAK OF EBOLA VIRAL DISEASE

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The outbreak of Ebola Virus Disease (EVD) in Liberia in 2014-15 infected about 10,675 and claimed the lives of more than 4,809 individuals. During this period the NTD, NCD and Eye health programmes were suspended. By June 2015 the EVD outbreak was under control, the Ministry of Health conducted an assessment of communities, health facilities and county health teams to understand their perception and readiness to resume programmes. The assessment was conducted in all counties including surveys of communities and health facilities affected and unaffected by EVD. All 36 communities surveyed reported they were ready and anticipating the resumption of programmes, especially MDA. All the communities desired increased engagement in the programme when it restarts as they recognized the benefits it presented. The survey also revealed disparities between individual programmes, though there is a high degree of acceptance of the lymphatic filariasis programme, the awareness and acceptance of the schistosomiasis programme was limited. Awareness for leprosy and Buruli ulcer is increasing but is limited. The number of trained personnel for these programmes, particularly at local health facilities, was found to be very low. This challenge has been exacerbated by high turnover of staff, during and following the EVD outbreak. Communities which were aware of one or more NCDs (22%) wanted treatment but felt they did not have access. Furthermore, eye care was very limited with 39% of communities reporting no access while the rest reported sporadic visitations by health workers or NGO groups over the last 5 years. The assessment highlighted the magnifying role that EVD had on the existing gaps within the NTD, NCD and Eye health programme, the desire within communities to resume NTD preventative treatment and also identified opportunities to build on the lessons learned during the outbreak for community mobilisation. The assessment also identified key recommendations related to leadership and governance, management and coordination, and planning and implementation to enable the programme to successfully move forward and increase its impact in post-EVD Liberia.

1209

EFFECT OF THE NATIONAL SCHISTOSOMIASIS CONTROL PROGRAM ON *TAENIA SOLIUM* TAENIOSIS AND PORCINE CYSTICERCOSIS IN RURAL COMMUNITIES OF TANZANIA

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Taenia solium is found throughout sub-Saharan Africa and co-endemic with schistosomiasis in many regions. *T. solium* leads to taeniosis and neurocysticercosis - the leading cause of preventable epilepsy globally. This study aimed to assess the effects of the National Schistosomiasis Control Programme (NSCP) on prevalence of *T. solium* and porcine cysticercosis over a four year period in Tanzania. School-based mass

drug administration (MDA) of praziquantel was carried out based on schistosomiasis endemicity. Four human and five porcine cross-sectional surveys were carried out from 2012 to 2015 in Mbozi and Mbeya district in Tanzania. Three rounds of school-based MDA of praziquantel were delivered in Mbozi and two in Mbeya by the NSCP. The prevalence of taeniosis and porcine cysticercosis was estimated annually. Stool samples were collected from humans and prevalence of taeniosis estimated by copro-Ag-ELISA. Blood samples from pigs were collected to estimate cysticercosis prevalence by Ag-ELISA. "Track and treat" of taeniosis cases were carried out after each survey. In total 12,082 stool samples and 4,579 porcine serum samples were collected. There was a significant higher prevalence of taeniosis in Mbozi compared to Mbeya prior to the intervention, but no difference at the end of the study. Significantly fewer children (≤15) from Mbozi were infected throughout the study than children from Mbeya who showed a significant decrease in copro-Ag prevalence after the first treatment only. During the final survey in Mbozi the prevalence of taeniosis in adults was significantly lower (p=0.031, OR 0.40, CI: 0.17-0.89), compared to baseline. The prevalence of porcine cysticercosis had also dropped significantly (p=0.002, OR 0.49, CI: 0.32-0.76) in this district compared to baseline, whereas no significant difference was seen in Mbeya. The study suggests that three rounds of MDA targeting schistosomiasis in school aged children contributed to a reduction in prevalence of infection with *T. solium* in this population, and also had a spill over effect on adults in treated areas as well as reducing the prevalence of *T. solium* in the pig population.

1210

USING A DOOR-TO-DOOR MASS DRUG ADMINISTRATION CAMPAIGN TO IDENTIFY TRICHIASIS AND HYDROCELE IN TOGO

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The Togolese Ministry of Health (MOH) utilized their highly successful Integrated Neglected Tropical Diseases (NTD) Control Program to identify and treat people with trachoma and hydrocele. Every year, Community Drug Distributors (CDDs) distribute medication to treat soil-transmitted helminths, onchocerciasis, and schistosomiasis. In 2015, the MOH received funding from the Bill & Melinda Gates Foundation to pilot a program to identify and treat individuals with trichiasis or hydrocele. CDDs identified persons with suspected trichiasis or hydrocele while conducting their drug distribution activities. A total of 5,665 suspected cases of trichiasis and 3,573 suspected cases of hydrocele were identified by CDDs nationwide. Funding for surgeries was limited, so case confirmation was performed first in the districts with the highest numbers of suspected cases. Trichiasis confirmation rates were low. Among the 3,269 cases reported in the Kara and Savanes regions, 2,143 were examined by ophthalmology technicians and only 17 (0.8%) were found to have trichiasis. The most common ocular issues identified were conjunctivitis (52%) and cataracts (17%). Sixteen of the 17 individuals with trichiasis were successfully treated, and one refused surgery. Among the 479 people with suspected hydrocele, surgeons examined 121, plus another 81 suspected cases that were identified in the field. A total of 202 suspected cases of hydrocele were examined, of which 101 were confirmed (50%). The most common finding among individuals who did not have hydrocele was hernia (99/101, 98%). All 101 individuals with confirmed hydrocele had surgical repair, with an average hospitalization period of eight days. Togo's integrated NTD drug distribution platform can be an effective means of identifying people in need of surgery for trichiasis and hydrocele. CDDs were better at identifying hydrocele than trichiasis, and better training of the CDDs is needed to help them correctly identify trichiasis. The approach should be repeated in subsequent years to identify additional individuals who warrant surgery.

1211

PROXY RESPONSES FOR MASS DRUG ADMINISTRATION COVERAGE SURVEYS: THE INDIVIDUALS REQUIRING THEM AND THE POTENTIAL FOR RECALL BIAS

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The success of mass drug administration (MDA) for neglected tropical diseases is dependent upon achieving adequate drug coverage, which is validated through household coverage surveys. Coverage surveys rely on respondent recall and often permit proxy responses, whereby another household member is allowed to respond on behalf of an absent individual. This study used data from coverage surveys for lymphatic filariasis in Malawi, Burkina Faso, and Uganda to determine the demographic characteristics of individuals for whom a proxy response was required and the extent to which a proxy response was associated with reported drug coverage. According to our results, teenagers and young adults (10 - 25 years) were more likely to be absent during the coverage survey and require a proxy response, compared with older adults (OR 1.96, p<0.001). Similarly, males were more likely to require a proxy response than females (OR 1.76, p<0.001). Adults who were eligible to receive MDA for lymphatic filariasis (i.e. everyone, excluding women who are pregnant or in the first week of breastfeeding and the severely ill) were more likely to be absent and require a proxy response than individuals who were ineligible for MDA (OR 2.86, p<0.001). A multivariate analysis found that individuals for whom a proxy response was provided had 1.52 times the odds of being recorded as having swallowed the drugs compared to self-reporting individuals, controlling for age and sex (95% CI (1.03, 2.24)). This finding is unexpected given that individuals who are unavailable at the time of a coverage survey may also have been unavailable at the time of MDA, and suggests that proxy respondents may be inflating drug coverage. While this finding could be explained in part by the fact that self-reporting individuals are more likely to have been ineligible to receive MDA and thus are expected to have lower coverage, this does not account for the entire increase in odds. This study highlights the possibility for recall bias in proxy responses to MDA coverage and suggests that further research is necessary to determine the best method for obtaining information on drug coverage when individuals are absent.

1212

FEASIBILITY AND SAFETY OF MASS CO-ADMINISTRATION OF AZITHROMYCIN AND IVERMECTIN MASS DRUG ADMINISTRATION: THE AIM STUDY

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Mass drug administration (MDA) has made a major contribution to the public health control of neglected tropical diseases across the world. The practical benefit of linking programs to increase efficiencies is well recognised, but limited in practice by uncertainties about the practical

and biological implications of co-administration of drugs. In the Solomon Islands, trachoma MDA with azithromycin was implemented in nine out of 10 provinces. When a decision was made to extend the program to the province of Choiseul, we had an opportunity to investigate the feasibility, safety and efficacy of co-administering ivermectin to control scabies and impetigo, diseases that were recognised as endemic in a number of countries of the Pacific region. The drug delivery infrastructure was established using the framework for trachoma. The MDA regimen was a single dose of oral azithromycin combined with a single dose of oral ivermectin. A second dose of ivermectin was given a week later to ensure elimination of scabies eggs that may have been present at first visit. Participants in 10 randomly selected villages were asked to undergo skin examination to collect scabies and impetigo baseline data. The study enrolled 26,188 participants, 99.3% of the total resident population. Of those, 98.2% received azithromycin and 98.5% received a first dose of ivermectin. A second dose of ivermectin was received by 83.7% of participants. In the survey villages, baseline scabies prevalence was 18.7% and highest in children aged 5-9 years (34%). Impetigo was present in 24.8% of participants, and highest in the 5-9 age group (46.4%). There were no serious adverse events. Adverse events were noted in 2.6% of the entire study population and 4.3% of participants in the more closely monitored skin survey sites. At present, this is the world's largest scabies MDA and the first large scale co-administration of Ivermectin and Azithromycin. Co-administration of ivermectin and azithromycin appears to be safe, well tolerated and feasible.

1213

ADDING TSETSE CONTROL TO MEDICAL ACTIVITIES ALLOWS CONTROL OF SLEEPING SICKNESS IN THE MANDOUL FOCUS (CHAD)

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Gambian sleeping sickness or HAT (human African trypanosomiasis) is a neglected tropical disease caused by *Trypanosoma brucei gambiense* transmitted by riverine species of tsetse. A global programme aims to eliminate the disease as a public health problem by 2020. The Mandoul area of Chad is a persistent focus of Gambian sleeping sickness where more than 100 HAT cases are still diagnosed and treated annually. Up to 2013, control of HAT relied solely on case detection and treatment, and did not lead to a clear and consistent decrease in the annual incidence of HAT despite annual screening of the population. We assessed whether the addition of vector control to case detection and treatment could reduce annual incidence of HAT in Mandoul. In particular, we investigated the impact of deploying 'tiny targets' which attract and kill tsetse. Before tsetse control commenced, a census of the human population was conducted and their settlements mapped. A pre-intervention survey of tsetse distribution and abundance was implemented in November 2013 and 2600 targets were deployed in the riverine habitats of tsetse in early 2014 and 2015. Impact on tsetse and on the incidence of sleeping sickness was assessed through six tsetse monitoring surveys and four medical surveys of human population in 2014 and 2015. The census indicated that a population of 26600 inhabitants lived in the vicinity of the Mandoul focus. Within this focus, the vector is *Glossina fuscipes fuscipes* and the mean catch of tsetse from traps was 0.7 flies/trap/day (range, 0-26). The catch of tsetse from 44 sentinel biconical traps declined after target deployment with only five tsetse being caught in five surveys giving a mean catch of 0.009 tsetse/trap/day. Simultaneously, HAT transmission declined from a mean of 127 cases/year between 2009 and 2013, to 52 cases in 2014 and only 25 new cases in 2015 with a similar medical effort.

1214

THE IMPACT OF MASS DRUG ADMINISTRATION ON REDUCTION OF NTD PREVALENCE IN RWANDA

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Worldwide an estimated 6 billion of the world's most impoverished people, including 875 million children are affected by Neglected Tropical Diseases (NTDs) which cause severe pain, long-term disability, and are the cause of death for over 500,000 people per year. In 2008, 65.8% of Rwandan schoolchildren were affected by Soil-Transmitted Helminth (STH) infections. Rwanda Ministry of Health in collaboration with its partners had started to implement large-scale NTD control through regular Mass Drug Administration (MDA) against these infections as per World Health Organization guidelines. Two national mapping surveys were conducted in 2008 and 2014 in order to assess schistosomiasis and STH prevalence at national level and geographic distribution. In 2008, a total of 8,313 schoolchildren aged between 10 and 17 years were tested for STH and schistosomiasis using Kato-Katz method. Prevalence of urinary schistosomiasis was established by testing for micro-haematuria using dipsticks and urine filtration technique. In 2014, a total of 9,250 schoolchildren aged between 8 and 18 years were tested for STH and schistosomiasis using Kato-Katz method while 19,371 schoolchildren were tested for schistosomiasis also using Circulating Cathodic Antigen (CCA) urine Assay. We carried out trend analysis for schistosomiasis and STH data from 28 schools that were randomly selected in both mapping surveys. All 28 schools are located in districts that reached at least 75% MDA therapeutic coverage for all treatment campaigns. For schistosomiasis, eleven (11) schools are located in areas that received praziquantel. Of these 11 schools 7 had 100% reduction in prevalence; three (3) had reduction between 39.4% and 93.0%. The comparison for STH infections showed a remarkable reduction in prevalence for only hookworm with 10 schools having 100% reduction and 17 schools with a reduction between 39.4% and 96.4%. These data demonstrate an encouraging quick impact of MDA in controlling schistosomiasis and STH and call for continuous support to NTD control programs of endemic countries.

1215

TARGETTING MALARIA HOTSPOTS IN SENEGAL: RESULTS OF A CLUSTER-RANDOMIZED TRIAL

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In Senegal scaling-up of control measures has reduced the incidence of malaria but transmission persists and new tools are needed to move towards elimination. We evaluated a targeted approach, employing IRS and chemotherapy implemented in transmission hotspots, on a large scale. 40 clusters (health posts) were randomized. In 30 clusters, hotspot villages were targeted to receive IRS with Actellic 300CS in July, followed

in 15 clusters by MDA with dihydroartemisinin-piperaquine (DHA-PQ) administered to all persons except pregnant women and children under 3 months of age, at the end of August and again in October. In the other 15 clusters, persons were screened using a malaria RDT and positives treated with DHA-PQ. 10 clusters served as controls. In all three arms, health promotion encouraged care seeking for fever, and a free LLIN was provided to each malaria patient at health facilities. The intervention strategy was delivered over two years. Primary outcomes were malaria incidence, and the prevalence of parasitaemia just after the main peak of transmission, in year 2. Adherence to treatment, and adverse events, were monitored after each round. Acceptability was investigated using in-depth interviews, and provider costs of the interventions were assessed. The year before intervention, malaria incidence was 11 per 1000, and parasitaemia prevalence by microscopy 1.9%. Interventions reduced annual incidence within hotspots by 46% (IRS+MDA) and by 52% (IRS+MSAT). Incidence in non-target communities within 2km of treated hotspots reduced by 41% (IRS+MDA) and 24% (IRS+MSAT). The overall efficacy against malaria (including target and non-target villages) was 37% (95% CI 31%,44%) in the IRS+MDA arm and 44% (38%,49%) in the IRS+MSAT arm. The strategies were well accepted and achieved high coverage. The cost of MSAT was 30% higher than for MDA. Adding IRS approximately doubled the cost. Where scaling-up of existing policies has reduced malaria transmission but additional measures are needed for elimination, targeted control with IRS and MDA or MSAT could be used to reduce transmission, but MDA was cheaper and slightly more effective than MSAT.

1216

COMPARISON OF MASS DRUG ADMINISTRATION VS. MASS SCREENING AND TREATMENT HIGH-RISK, MILITARY MOBILE POPULATIONS TO SUPPORT MALARIA ELIMINATION IN CAMBODIA

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The Cambodian government has set a goal for malaria elimination by 2025. However, the effectiveness of elimination strategies in hard-to-reach mobile populations, including the military, is largely unknown. We are conducting a two-arm, controlled, cluster-randomized, open-label pilot study to determine the effectiveness of monthly malaria prophylaxis (MMP), using dihydroartemisinin-piperaquine (DP) and weekly primaquine (22.5 mg), compared to monthly focused screening (microscopy and PCR) and treatment (FSAT) of malaria positive subjects according to the national treatment guidelines. After 3 months of interventions, both arms will be actively followed for 3 more months to assess malaria incidence in the rainy season when malaria usually peaks. Of 1,114 active duty military and dependents screened in Oddor Meanchay province near the Thai-Cambodian border, 1,050 volunteers were enrolled into 8 clusters. We noted reductions of malaria incidence within 2 months in both arms from a baseline prevalence of 53/516 (10.3%; 39 cases of *Plasmodium falciparum* (*P.f)*, 13 cases of *P. vivax* (P.v) (*P.v,* 1 cases of mixed) and 91/534 (17.0%; 45 cases of *P.f*, 38 cases of *P.v,* 8 cases of mixed) malaria positive at screening in the FSAT and MMP arms, respectively. In the first month, 8/509 (1.6%) subjects from the FSAT arm and 2/529 (0.4%) from the MMP arm had a *P. falciparum* requiring an unscheduled visit, with an additional 10/509 (1.9%) in the FSAT and 16/529 (3.0%) in the MMP being malaria positive on the day 30 follow-up visit (p=0.906). On day 60 follow up, only 6/489 (1.2%; 0 cases of *P.f* and 6 cases of *P.v*) and 2/504 (0.4%; 1 cases of *P.f*, 0 cases of *P.v*) subjects were malaria positive by microscopy or PCR in FSAT and MMP arms, respectively, showing low parasitemia in both treatment arms (p=0.172), reaching statistical significance by month 3 follow-up, with 20/472 and 0/502 cases of malaria in FSAT and MMP arms, respectively (p<0.001). The number of subjects withdrawn or lost to follow up remains low at around 3% in each arm. Most malaria cases in the MMP arm occurred within 1 month of follow-up and likely represent *P.f* treatment failures of DP. Final outcome data from 6 months of follow-up will be presented.

1217

RELATIVE CONTRIBUTION OF GENERALIZED EARLY DIAGNOSIS AND TREATMENT AND OF TARGETED MASS TREATMENT TO ELIMINATION OF *PLASMODIUM FALCIPARUM* MALARIA IN EASTERN MYANMAR

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Plasmodium falciparum (PF) malaria elimination is on the agenda of 19 countries. In the Greater Mekong Sub-region, elimination is of particular interest and urgency because of the threat of spreading artemisinin resistance. In coordination with community-based health organizations and the Myanmar National Malaria Control program, the Malaria Elimination Task Force was set up to develop strategies and to implement a regional approach towards PF elimination in 4 districts of Eastern Myanmar. Malaria Posts (MP) were deployed in each community of the target area to provide access to early diagnosis and treatment of malaria. MP reported PF and *P. vivax* (PV) case data weekly. Village-level malaria prevalence was measured in surveys analyzed by ultrasensitive qPCR. Hotspots of asymptomatic malaria prevalence were defined by malaria prevalence > 40% with PF representing >20% of all malaria infections. Hotspots were addressed by 3 rounds of targeted mass treatment (TMT) using dihydroartemisin-piperaquine. A generalized linear mixed model adjusting for season and location was used to analyze PF case counts, monitor trends in PF incidence and determine the relative contribution of MP and TMT to malaria elimination. From May 2014 to April 2016, >800 villages were equipped with MP and reported weekly data. Out of 218 surveys performed, 43 hotspots were identified and addressed with 3 consecutive months of TMT between January 2015 and March 2016. The probability of an MP declaring ≥1 PF case during its first month of operation was 26% and decreased to below 10% after 18 months, while the probability of declaring ≥1 PV case remained stable around 30%. The PF incidence rate ratio (IRR) for 10 additional weeks of MP operating in a village was 0.78 (95%CI=0.74-0.82). Before TMT IRR for hotspot villages compared to non-hotspot villages from the same area was 2.6 (95%CI=1.3-5.0). After TMT incidence in hotspot villages was similar to non-hotspot villages (IRR=1.4; 95%CI=0.6-3.5). Over 24 months of follow-up, the deployment of an MP network in 4 districts triggered a strong decrease of PF incidence rate. TMT proved to accelerate the decrease in high prevalence villages.

1218

IMPACT OF MASS DRUG ADMINISTRATION WITH DIHYDROARTEMISININ-PIPERAQUINE ON MALARIA TRANSMISSION IN A HIGHLY SEASONAL TRANSMISSION SETTING IN THE GAMBIA

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There is renewed interest in mass drug administration (MDA) as a strategy to interrupt malaria transmission in settings approaching elimination. Data on its impact in areas with seasonal and heterogeneous transmission are limited. The objective of the study is to evaluate the impact of MDA with Dihydroartemisinin-Piperaquine (DHA/PPQ) on *Plasmodium falciparum* carriage in low-moderate transmission settings in The Gambia. In June 2014, the start of the transmission season DHA/PPQ was administered using weight based dosing to residents between ≥6 months and <75 years of age in twelve villages across the five regions of the country. A baseline community survey was conducted prior to MDA. Participants were followed up with monthly surveys from July to December. Finger prick blood samples were collected for parasite detection using diagnostic PCR. A total of 3888/4677 (83.1% of the population) were eligible and received DHA/PPQ. All pregnant participants or elderly participants over 75 years were excluded (4.2%, 172/4060). The prevalence of *P. falciparum* post-MDA was 5.43% (170/3130; 95%CI 4.6-6.2%). The villages in the eastern region where transmission is moderate had a 44.03% reduction in prevalence (12.9%, 327/2527 in 2014 vs 23.1%, 1185/5141 in 2013) while in the rest of the country where transmission is low villages in the central and western regions there was a 17.0% reduction in infection prevalence 3.9% (81/4691) in 2014 and 4.6% (637/13727) in 2013. Preliminary results show, the overall incidence rate of *P. falciparum* parasitemia was 0.2 (95%CI: 0.2-0.2) episodes per person-year at risk in the 2014 transmission season. The risk of *P. falciparum* decreased with increase in age; children 5-15 years (HR=1.7, 96%CI: 1.3-2.2) and adults 15-29.9 years; HR=1.4, 95%CI: 1.10-1.9). The risk of infection increased with severity of anaemia (severe anaemia; HR=3.1, 95%CI: 1.6-6.4). The most significant impact of MDA on *P. falciparum* carriage was in communities located in areas with moderate transmission compared to areas with very low prevalence. MDA might be considered as an additional intervention in areas of on-going moderate malaria transmission.

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FREEDOM FROM INFECTION: MEASURING THE INTERRUPTION OF MALARIA TRANSMISSION

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Good malaria surveillance is the foundation for effective malaria elimination efforts and should generate timely and actionable information for decision makers. Measuring elimination poses a specific challenge in that it involves proving a negative: when a country shifts into the scenario of zero-malaria reporting, improved strategies are required for identifying areas where there is more or less certainty in having achieved elimination. Here, we present quantitative tools by which the probability of having achieved freedom from malaria infections can be calculated. These tools are applied to the malaria context in order to provide accessible and actionable tools for countries engaging in malaria elimination and are evaluated against the current WHO guidelines. Assuming a single population of 100,000 people (i.e. a health facility catchment area), reporting zero cases per month over three years, results suggest we can achieve a 95% probability that there are in reality fewer than 100 cases. We can be sure that there are fewer than 50 cases with a probability of 80% or 95% in 5 or 15 years of monthly zero reporting, respectively if relying solely on passive surveillance. To improve the probability of freedom achieved within 3 years, programs have the option for supplementing passive surveillance with active 'freedom' surveys that will increase the system sensitivity for that reporting month. To achieve a high probability of achieving freedom within 3 years, at least 2 population-based 'freedom surveys'. Therefore, the freedom tools can be used for estimating the level of confidence that has been achieved in asserting a malaria-free status and for informing programs where and how to target efforts to boost to the desired level of certainty that elimination has been achieved. Such an approach is particularly important in settings that are accelerating the timeline to elimination where it is paramount to measure the absence transmission on a shortened timeline.

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THE NATURAL HISTORY OF MALARIA ELIMINATION IN SOUTHERN ZAMBIA

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As areas transition from malaria control to elimination, targeted interventions will be needed. Knowledge of changing spatial and temporal patterns of transmission and parasite genetic structure and divergence can guide elimination efforts. This study describes the natural history of malaria elimination in the catchment area of Macha Hospital in Choma District, Southern Province, Zambia. Passively-detected, symptomatic cases of malaria were reported from six rural health centers from August 2008 to September 2015. Seasonal malaria incidence was estimated for each catchment area and spatial patterns were evaluated. Asymptomatic, infected individuals were identified through active case detection using rapid diagnostic tests and PCR for *Plasmodium falciparum* in population-based, longitudinal and serial cross-sectional cohort studies conducted from February 2008 to October 2013. Spatial clusters of asymptomatic cases were estimated and seasonal overlap was assessed. A 24 single nucleotide polymorphism molecular barcode was used to determine the genetic relationship between *P. falciparum* parasites from symptomatic and asymptomatic cases. Genetic relatedness was analyzed using phylogenetic trees and genomic divergence was calculated for each season. After the 2009-2010 transmission season, the incidence of symptomatic cases shifted from an annual to a biannual pattern and parasite prevalence by active case detection decreased substantially. A fractured spatial pattern was observed for symptomatic cases after the 2010-2011 transmission season. Phylogenetic trees showed independent clustering of symptomatic and asymptomatic cases, suggesting asymptomatic infected individuals were not contributing substantially to on-going transmission. Genetic complexity was high in asymptomatic cases, consistent with a chronically-infected reservoir, and low in symptomatic cases. Parasite genetic divergence decreased among asymptomatic cases, indicating loss of parasite diversity in this chronically-infected reservoir, but remained high in symptomatic cases, consistent with some degree of parasite importation.

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GENOMIC TOOLS REVEAL TRENDS IN *PLASMODIUM FALCIPARUM* PARASITE DIVERSITY ACROSS TRANSMISSION GRADIENTS WORLDWIDE

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Recent progress in malaria control and elimination has underscored the need for development of new tools and methodologies to assess progress towards programmatic milestones and signal program reorientation. The renaissance of genomic approaches to complement epidemiological tools has offered unparalleled opportunities to better assess the parasite population underlying characteristic epidemiological signals, such as transmission intensity. However, there are few data relating the signals of parasite diversity to transmission intensity levels from global populations. The current study assessed the correlation of genetic-based signals of parasite populations to determine their usefulness as a proxy for relative transmission levels. *Plasmodium falciparum* complexity of infection (COI), relatedness, and clonality from a worldwide population of parasites from Southeast Asia (Bangladesh and Cambodia), the Americas (Panama, Peru, and Haiti), and Africa (Zambia, Mozambique, Malawi, Burkina Faso, Uganda, and Senegal) were assessed using the molecular barcode. The results of this analysis showed clear trends in parasite populations along the gradient of transmission intensity, both within and between countries. Samples from regions with relatively low intensity, such as those from the Americas, showed increased clonality and a clear ability to track individual parasite lineages during epidemic outbreaks. In contrast, areas with high transmission had reduced clonality and overall relatedness compared to low transmission settings. Furthermore, sampling of 6 surveillance sites within Senegal also demonstrated that relative transmission intensity was associated with characteristic population diversity, as well as several instances of parasite lineages shared between regions, suggestive of importation. The findings of this study support the usefulness of genomic tools such as the molecular barcode for programmatic surveillance. These tools require sampling a small number of individuals to provide real-time, informative data with operational applications and the potential to impact national and regional policies.

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HOST EXPOSURE TO EARLY LIFE STAGES OF *SCHISTOSOMA HAEMATOBIUM* DOES NOT ALTER THE BLADDER RESPONSE TO EGGS

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Despite the significant impact of urogenital schistosomiasis (*Schistosoma haematobium* infection) in endemic regions, relatively little is known regarding the pathophysiology of this disease. Primate models remain a powerful tool to approximate human schistosomiasis, yet many technical reasons prevent widespread use of these models, including high costs, low availability, and ethical considerations. Infecting rodents percutaneously with *S. haematobium* cercariae, the route of natural human infection, results in low or no involvement of the urogenital system. Previously, we have developed a mouse model of urogenital schistosomiasis that bypasses this limitation via microinjection of viable *S. haematobium* eggs directly into the bladder wall. This model recapitulates many relevant sequelae found in human disease, including granuloma formation, urinary tract fibrosis and dysfunction, and systemic Th2 responses. However, it is unclear whether the absence of host exposure to the early parasite life stages (cercariae, schistosomules, and worms) prior to egg deposition in the bladder affects the resulting systemic and local immune response. We addressed this question by comparing mice that underwent bladder wall injection with *S. haematobium* eggs to mice that were percutaneously infected with *S. haematobium* cercariae followed 8 weeks later by microinjection of their bladder walls with *S. haematobium* eggs. Portions of these mouse cohorts were also treated with praziquantel following bladder wall injection. Based on worm counts, histology, and cytokine analyses, we conclude that while subtle differences exist, bladder wall injection with *S. haematobium* eggs alone may be sufficient to model urogenital schistosomiasis in mice. These findings also have implications for understanding chronic egg-induced bladder inflammation in people who have been cured of schistosomiasis by drug therapy and/or acquired immunity. Namely, the absence of continued antigenic stimulation by early parasite life stages may not impact ongoing bladder inflammation triggered by previously deposited *S. haematobium* eggs.

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MATERNAL *SCHISTOSOMA MANSONI* INFECTION ALTERS THE IMMUNE RESPONSE OF OFFSPRING TO TETANUS AND DIPHTERIA IMMUNIZATION

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Maternal helminth infections have been implicated in the reduced efficacy of critical life saving neonatal and childhood vaccines. Approximately 40 million women of childbearing age are at risk for schistosomiasis and at least 10 million women in Africa have schistosomiasis during pregnancy, yet little is known about the impact of prenatal exposure on the immune responsiveness of offspring. Findings from previous studies indicate that a fetus can be exposed to helminth antigens *in utero*, so the high frequency of schistosomiasis in this population represents a significant potential public health problem. The underlying mechanisms through which maternal infection with helminths, such as *Schistosoma mansoni*, function to negatively impact neonatal vaccine responses, however, remain to be discovered. We sought to determine the immunological mechanism(s) through which responses to immunization with tetanus/diphtheria are altered following prenatal *S. mansoni* infection in an experimental mouse model of *S. mansoni* infection. Interestingly, offspring from infected mothers exhibited smaller germinal centers after immunization with Tetanus/Diphteria than pups born to uninfected mothers. In addition,

the offspring from infected mothers presented decreased numbers of IL-4 secreting TFH cells and decreased expression of B cell co-stimulatory markers in comparison to age-matched offspring of uninfected mothers. These profound alterations suggest a possible mechanism for the previously reported reductions in vaccine specific titers in offspring born to helminth infected mothers in endemic countries.

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RECOMBINANT PARAMYOSIN IN MONTANIDE ISA 206 PROTECTS WATER BUFFALO FROM *SCHISTOSOMA JAPONICUM* INFECTION

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Schistosomiasis japonica is a zoonosis and a major disease risk for more than 40 million people in China and 7 million more in the Philippines. Because water buffalo play a critical role in transmission to humans, a veterinary vaccine would have a significant impact on prevalence and incidence of human infection while also improving animal health and economic output. Paramysin (Sj97) is a 97 kDa myofibrillar protein with a coiled-coil structure found only in invertebrates. We successfully produced pilot-scale recombinant full-length paramyosin (rSj97) and assessed its safety and efficacy in vaccine challenge experiments in water buffalo. A three-dose regimen with 250 or 500 mg rSj97/dose in ISA206 was well tolerated with no severe adverse events, no differences in body condition score and no differences in serum chemistry assays compared to controls. In our first challenge trial, buffalo were immunized three times at 4-week intervals with 250 mg /dose rSj97 in ISA206 (n=7) or ISA206 alone (n=6). Buffalo were challenged with 1,000 *S. japonicum* cercariae and perfused 8 weeks post challenge. Worm burdens were reduced by 51.5%, but this reduction did not achieve statistical significance, likely due to small sample size. In a second vaccine-challenge experiment of similar design, buffalo immunized with 500ug/dose of rSj97 in ISA206 had 60.9% lower worm burdens compared to adjuvant controls (p=0.05). A similar reduction (57.8%) was observed with animals immunized with 250ug rSj97/dose. Egg recovery from liver tissue was positively correlated with worm burden (R^2=0.4876, p<0.001), while anti-rSj97 specific IgG_2 titers at 4 weeks after 3rd immunization were negatively correlated with worm burden (R^2= -0.275, p=0.03). Moreover, rSj97 stimulated markedly increased IFN-gamma levels in whole blood cultures from rSj97-ISA206 immunized animals compared to controls. These findings indicated that rSj97 is a safe and promising schistosomiasis veterinary vaccine and mandate further evaluation of rSj97 based vaccine safety and efficacy.

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A MICROFILTRATION DEVICE FOR DIAGNOSIS OF UROGENITAL SCHISTOSOMIASIS

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Urogenital schistosomiasis, infection of the urinary tract by *Schistosoma haematobium* worms, is a cause of urologic disease in over 112 million people worldwide. The diagnostic standard of care, counting eggs in urine, is slow, making it difficult to use as a point-of-care test for infection. This is a major barrier to mass drug administration campaigns seeking to eliminate *S. haematobium*. Herein we describe the development of a microfiltration device for trapping, isolation, and on-chip fluorescence characterization of schistosome eggs toward rapid diagnostics of urogenital schistosomiasis. The device comprises a linear array of microfluidic traps to immobilize and separate eggs of *S. haematobium* from urine. The trap array allows sequential loading of individual eggs by flow resistance to facilitate observation and enumeration of samples with low-abundance targets. Computational fluid dynamics modeling and experimental characterization are performed to optimize the trap design for enhancing the trapping efficiency and throughput. The microfiltration device is capable of isolating schistosome eggs from urine and the trapped eggs can be recovered for downstream analysis. The trapping efficiency of the device is 100% with 300 µl/min and 83% with 3000 µl/min. The filtration procedure can be finished within 10 min. The microfiltration device is capable of isolating schistosome eggs from urine and the trapped eggs can be recovered for downstream analysis. On-chip staining is demonstrated in the microfiltration device for fluorescence analysis of schistosome eggs. In conclusion, our results are proof of concept that a microfluidics device can be used to more efficiently trap and separate *S. haematobium* eggs in urine. Further optimization of this device may lead to a point-of-care diagnostic suitable for use in the field.

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AN EGG PROTEIN FROM *SCHISTOSOMA MANSONI* IS PROMISING FOR DEVELOPMENT OF HIGH-SPECIFICITY DIAGNOSTICS IN THE ERA OF INTENSIFIED CONTROL

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Schistosomiasis is a serious global public health problem. The standard for diagnosis is the Kato-Katz method, which has low sensitivity and therefore does not work well on patients with low-level infections, representing the majority of cases. Adding tests such as ELISAs using soluble egg antigens (SEA), increases diagnostic accuracy in low burden areas of Minas Gerais, Brazil. However, crude SEA antigens have low-specificity and cross-react with other helminthes. Therefore, the goal of this work is to identify SEA proteins with high schistosomiasis specificity, as well as the sensitivity to differentiate between active (acute and chronic) and cured (post-treatment phase) infections, in order to develop point-of-care (POC) tests as well as improve others diagnostic methodologies. To complete these studies, SEA was generated from mice 45 days after infection with *Schistosoma mansoni*. Using a protocol approved by the Brazilian Ethical Committee, human serum was obtained in Minas Gerais from each group: healthy volunteers (negative controls); schistosome acute, chronic and post-treatment patients; and patients infected with other helminthes. The sample from each group were submitted to two-dimensional Western blot (2D-WB) using native and sodium metaperiodate (SMP) treated SEA. The immunoreactive spots were identified by mass spectrometry and analyzed by bioinformatics tools. A total of 23 spots were identified by serum from Schistosoma infected patients. Among these, 22 spots were identified by serum from patients infected with other helminths, and 9 by negative control samples. One spot was uniquely recognized by sera from Schistosoma-infected patients and detection remained after sugar denaturation by SMP treatment, suggesting serum antibodies were binding to peptide epitopes. We identified a potential egg protein from this unique spot, for which we are developing monoclonal antibodies, toward the development of POC immunodiagnostic test and highly specific ELISAs. Further, the fast, simple POC assay requires minimal equipment and will be an accurate screening tool for epidemiologic surveying in low resource regions.

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MAPPING OF SCHISTOSOMIASIS IN RWANDA: USE OF POC-CCA VERSUS KATO-KATZ

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Worldwide an estimated 240 million of the world's most impoverished people are affected by schistosomiasis. Classic severe schistosomiasis-related morbidities include periportal fibrosis in intestinal schistosomiasis caused by *Schistosoma mansoni* and *S. japonicum*, and bladder deformity and hydronephrosis caused by *S. haematobium*. However, the more subtle, but nonetheless disabling morbidities of anemia, growth stunting, and, cognitive impairment likely represent the wider public health challenge. In 2008, the Rwanda Ministry of Health in collaboration with its partners began implement large-scale NTD control, including schistosomiasis, through regular mass drug administration (MDA) against these infections as per WHO guidelines. A national NTD mapping survey was carried out from June to mid-July 2014, using both the Kato-Katz and the Point-Of-Care Circulating Cathodic Antigen (POC-CCA) assays for detection of *Schistosoma mansoni*. Fifty children of the required age (primarily 13-14 years) were randomly selected in each of 399 schools. For diagnosis of *S. mansoni* duplicate fecal Kato-Katz thick smears and the urine POC-CCA test were performed. A total of 19,934 pupils were included, with 19,371 from 388 schools tested with POC-CCA and 9,250 from 186 schools tested with Kato-Katz. There was an approximately equal number of boys and girls sampled in each school. Ages of the sampled children ranged from 8 to 18 years, although 88% of children sampled were 13 or 14 years, and 98% of children were in the range of 10-14 years. When POC-CCA Traces readings were considered negatives, overall schistosomiasis prevalence was 7.4% (95%CI: 5.7%-9.5%) with 0.6%-16.7% as range in mapping units; when POC-CCA Traces readings were considered positives, overall schistosomiasis prevalence was 36.1% (95%CI: 32.6%-39.8%) with a range of 10.8%-63.8% in mapping units; With Kato-Katz overall schistosomiasis prevalence was 1.9% (95%CI: 1.1%-3.3%) with a range of 0.0%-9.4% in mapping units. Results from this mapping survey demonstrate issues in the use of POC-CCA in the implementation of national program and the need for related WHO guidelines.

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A NOVEL CELL FREE DNA DETECTION ASSAY FOR THE DIAGNOSIS OF SCHISTOSOMIASIS JAPONICA

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Accurate diagnostics play a pivotal role in achieving current schistosomiasis control and elimination goals. Lack of accuracy and inability to detect pre-patent infections are major limitations of current diagnostic procedures. We have optimised a novel droplet digital PCR duplex assay for the diagnosis of schistosomiasis japonica which provides improved detection sensitivity and specificity. The assay involves the amplification of two specific and abundant target gene sequences in *Schistosoma japonicum*; a retrotransposon (*SjR2*) and a mitochondrial gene (*nad1*). The assay detects target sequences from different schistosome lifecycle stages (adult worms, schistosomules and eggs) and exhibits a high level of specificity, representing an ideal tool for the detection of low levels of parasite DNA in different clinical samples. Following optimization, the assay was validated using a *S. japonicum*-infected mouse model. It detected both pre-patent and patent infections using serum, urine and faecal DNA collected at different time points. There was a positive correlation between the gene copy numbers and the intensity of infection determined by egg and worm counts. The assay is quantitative and can be used to determine parasite infection intensity and will be an important diagnostic tool, particularly for the detection of low intensity infections in low prevalence schistosomiasis-endemic areas.

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MATURATION OF FLAVIVIRUSES AND ROLE IN DISEASE

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Many viruses are initially assembled in host cells in a non-infectious immature form and are converted to their mature infectious state through a series of coordinated events requiring specific triggers. This prevents premature disassembly of these viruses during egress from host cells. For flaviviruses, the maturation process involves pH-driven structural rearrangements of precursor Membrane (prM) and Envelope (E) glycoproteins and furin protease-mediated cleavage of prM during transit through the trans-Golgi network. Despite the cleavage of prM being crucial for virus infectivity, this process is inefficient for dengue virus (DENV). Virus preparations comprise a continuum of particles with variable maturity from immature 'spiky' (having high prM content) to fully mature 'smooth' particles (having low or zero prM content) determined using cryo-electron microscopy. The consequence of inefficient maturity is structural heterogeneity that may be advantageous for the virus for immune evasion and pathogenesis. We have developed sensitive biochemical and biophysical tools to quantitate the maturity of DENV and other flaviviruses and explore the role of prM in virus dynamics, disease pathogenesis and vaccine design/quality control. We have developed a mass spectrometry-based selected reaction monitoring assay and use it in tandem with cryo-electron microscopy to probe prM content/ maturity of different serotypes of DENV produced in cell culture and are currently developing protocols to study virus produced from natural infection of mosquitoes (vector) and humans (host). Using these methods, we have detected differences in the cleavage efficiency of prM among different serotypes of DENV that have implications in virus breathing, structural dynamics and specific infectivity. These studies have been broadened to examine this cleavage and its effect on virus structure using other flaviviruses.

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FLAVIVIRUS NS1 PROTEINS DIFFERENTIALLY INDUCE HYPERPERMEABILITY IN HUMAN ENDOTHELIAL CELL MONOLAYERS FROM DISTINCT TISSUE TYPES

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Dengue (DENV), Japanese encephalitis (JEV), West Nile (WNV), yellow fever (YFV) and Zika (ZIKV) viruses belong to the *Flavivirus* genus, *Flaviviridae* family. Severe dengue disease is characterized by increased vascular leakage leading to pleural effusion, causing hypotension and death. In contrast, WNV and JEV infections can result in neurological diseases, including encephalitis. ZIKV appears to cause pathology in both the placenta and the fetal brain. Evidence suggests that disruption of physiological barriers such as the microvascular endothelium or the blood brain barrier can play a critical role in flavivirus pathogenesis. Recently, we described a direct effect of DENV non-structural protein 1 (NS1) in triggering endothelial barrier dysfunction in human pulmonary

microvascular endothelial cells (HPMEC) *in vitro* and systemic vascular leakage *in vivo*. Here, we examined the ability of flavivirus NS1 proteins to interact and modulate endothelial barrier function of different endothelial cell (EC) types: HPMEC, human dermal EC (HMEC-1), brain microvascular EC (HBMEC), and umbilical vein EC (HUVEC). We show that flavivirus NS1 proteins interact with the surface of human EC and alter the barrier function, as measured by Trans-endothelial Electrical Resistance (TEER), in a cell-type dependent manner. DENV2 NS1 induced permeability in all endothelial cell types tested. However, WNV and JEV NS1 only significantly reduced TEER of HBMEC. ZIKV increased permeability only in HUVEC. YFV NS1, a flavivirus that causes severe hepatic injury, moderately increased permeability of only HPMEC. Ongoing TEER experiments using hepatic endothelial cells will further explore this specificity. These *in vitro* EC permeability results appear to reflect the tropism of the disease caused by each virus. Our findings propose a mechanism by which flavivirus NS1 proteins differentially cause endothelial barrier dysfunction, potentially resulting in increased plasma leakage, inflammation, or virus dissemination through different biological barriers as a part of the flavivirus pathogenesis process.

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DISCOVERY OF SMALL MOLECULE BIOMARKERS THAT PROVIDE METABOLIC SIGNATURES TO DIFFERENTIATE DENGUE, CHIKUNGUNYA AND ZIKA VIRUS INFECTIONS AND DENGUE DISEASE SEVERITY

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Chikungunya (CHIKV) and Zika viruses (ZIKV) are now co-circulating with dengue viruses (DENV) in over 30 countries in the Americas. Accurate diagnoses and prediction of disease severity using acute phase clinical specimens is critical for appropriate triaging and better clinical management of diseases caused by these viruses. We have developed a versatile metabolomics platform to identify small molecule biomarkers (SMBs) in serum that predict dengue disease severity and are currently defining metabolic signatures that differentiate the three arboviral diseases. This platform combines reverse phase ultra-high performance liquid chromatography-mass spectrometry (RP-UPLC-MS) and hydrophilic interaction liquid chromatography (HILIC) -MS and was used to characterize the acute phase serum metabolome of patients from Nicaragua and Mexico who were diagnosed with different grades of dengue disease severity, dengue fever (DF), dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS). Patients with laboratory-confirmed infections with DENV, CHIKV, or ZIKV contributed serum, urine and saliva samples during the 2015/2016 transmission season, and we have used these samples to validate SMBs for dengue diagnosis and prognosis and explore similarities and differences unique to CHIKV and ZIKV infections. We will discuss the biochemical classes of metabolites identified by the combined platform and highlight SMB signatures that are specific for each disease type. Metabolic markers that are specific to flavivirus (DENV and ZIKV) infections versus an alphavirus (CHIKV), or differentiate between the flaviviviruses could potentially provide important information on differential pathogenesis and could be exploited for development of therapeutics. These comparative studies are particularly important, as the clinical presentation of these diseases in the acute phase can be similar.

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HUMAN DENV2 MONOCLONAL ANTIBODIES TARGET MULTIPLE EPITOPES ON THE ENVELOPE GLYCOPROTEIN

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Dengue virus (DENV) infection causes dengue fever and more severe disease, and it is estimated that a third of the world is at risk for infection. Dengue serotype 2 (DENV2) is widespread, causes severe epidemics, and the recently licensed live tetravalent dengue vaccine was not protective against DENV2, despite protecting against the other three serotypes. DENV2 infections stimulate durable, serotype-specific neutralizing and protective antibodies in people. Previous work from our group has shown that the majority of DENV2 type-specific neutralizing polyclonal antibodies targets a complex epitope containing EDIII of the envelope (E) glycoprotein. While the crystal structure of a single human DENV2 type-specific neutralizing antibody, 2D22, has been solved, it was not known if this epitope is representative of a diverse panel of DENV2 type-specific antibody epitopes. Using a DENV infectious clone system, we have generated a panel of recombinant DENV viruses that were then used in binding and neutralization assays. With these experiments we have mapped the epitopes of additional DENV2 type-specific neutralizing antibodies. While some of these antibodies share a similar epitope to 2D22, others use entirely different domains in their epitopes. Our results reveal that there are multiple epitopes targeted by DENV2 neutralizing antibodies. Future work aims to determine what the proportion of each of these antibody epitopes are in polyclonal immune sera, and test whether antibodies targeting one epitope is more important for neutralization that the other.

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USE OF THE DENGUE HUMAN CHALLENGE MODEL TO CHARACTERIZE THE CLINICAL AND IMMUNOLOGICAL RESPONSE TO SEQUENTIAL HETEROTYPIC DENGUE INFECTION

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Dengue is recognized as the most important mosquito-borne virus worldwide. There are 4 dengue viruses (DENV), each capable of causing the full spectrum of disease which ranges from an asymptomatic or mildly symptomatic infection to a live-threatening illness characterized by vascular leak syndrome. Epidemiologic studies have demonstrated that the greatest risk for developing severe dengue occurs with a second, heterotypic DENV infection. This is thought to be mediated by antibody-dependent enhancement of infection in which antibodies from the primary DENV infection opsonize the secondary, heterotypic DENV but instead of neutralizing the virus, the antibody-virus complex is able to enter target cells through the FcγR2a leading to higher titers of virus. Interestingly, third and fourth DENV infections appear to result in less severe illness. Using our controlled dengue human challenge model, we studied the clinical and immunological response to sequential heterotypic DENV infection to characterize the neutralization and enhancement qualities following primary and secondary dengue infection. Twelve flavivirus-naïve subjects were enrolled and randomized to receive the live attenuated DENV-1 DEN**α**30 (n=9) or placebo (n=3). Nine months later 8 subjects were received the DENV-2 challenge virus DEN2**α**30 (6 had received DEN1**α**30; 2 had received placebo). The treatment assignments remained blinded until

study day 360. Following primary infection, 9/9 subjects were viremic with DENV-1 (mean peak titer = 0.9 $\log_{10}$ PFU/mL), 8/9 seroconverted to DENV-1, 2/9 seroconverted to DENV-2 NGC, 4/9 seroconverted to DENV-3, and 0/9 serconverted to DENV-4. Antibody to DENV-2 decreased to <1:5 by study day 90. Following challenge with DENV-2 9 months later, 6/6 DENV-1 recipients had detectable DENV-2 viremia and 3/6 had rash compared with 2/2 controls with detectable viremia and rash. The virologic and immunologic responses following primary and secondary DENV infection will be discussed.

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HLA DRB1 ALLELIC VARIANTS ARE ASSOCIATED WITH DIFFERENT RESPONSE MAGNITUDE OF DENV SPECIFIC CD4 T CELL RESPONSES AND DISEASE SEVERITY OUTCOMES

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All four dengue virus serotypes are now simultaneously circulating worldwide being responsible for 400 million human infections each year with around 100 million resulting in apparent infections. The observation that only a minority of patients develops severe disease suggests that host genetic factors may play an important role in disease severity. HLA molecules that restrict CD4 and CD8 T cell responses are one of the most polymorphic host factors in humans with several thousand variants thus far known. While DENV-specific CD8+ T cell responses have been extensively studied, the breadth and specificity of CD4+ T cell responses remains to be defined. Here we map CD4 T cell responses in individuals previously exposed to dengue virus. To identify HLA class II candidates, we utilized a panel of algorithms for sixteen common HLA DR molecules representative of the main DR supertypes. These efforts led to the identification of 365 epitopes derived from all 10 DENV proteins. In contrast to CD8 T cell targets, the highest number of epitopes was associated with the structural capsid protein (C), followed by nonstructural NS3, NS2A, NS5 and envelope proteins (E). Similar to CD8 T cell responses we noticed a wide variation in magnitude of T cell responses as a function of the restricting DRB1 allele. To investigate if HLA specific variations in magnitude of response might actually predict as yet unknown associations between DENV disease outcomes and HLA DRB1 alleles we assembled a set of 411 samples from hospitalized patients with confirmed diagnosis of Dengue fever or dengue hemorrhagic fever. We found several HLA alleles that were associated with stronger responses and showed significant OR<1 indicating a lower risk of hospitalized disease (protective effect). Phenotyping of the responding CD4+ T cell subsets revealed a DENV specific T cell subset, specifically expanded in donors carrying an allele associated with protection from severe disease and which is absent in DENV negative donors. Detailed knowledge of phenotype and function of DENV-specific T cells will aid in the establishment of correlates of protection against severe disease.

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ORGAN DAMAGE AND IMMUNOPATHOLOGICAL FEATURES IN FATAL DENGUE CASES

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Dengue is the most common arthropod borne infectious disease worldwide. The infection may be asymptomatic or mild, some subjects develop severe dengue with hemorrhagic manifestations, fluid leakage and organ failure leading to death. The extent of organ involvement in disease and death is not clear. A systematic analysis of the immunohistopathological reactions of these organs will provide important clues on the pathogenesis of severe dengue. 50 fatal dengue cases which underwent autopsy between 2010 and 2015 were analyzed. Most of them positive for dengue by RT-PCR in tissue. FFPE tissues from liver, lung, heart, kidney, brain, spleen, and lymph node were stained with H&E and immunostained for T cells, CD4+ and CD8+T cells, macrophages and dengue antigen. Vascular congestion, hemorrhagic changes and inflammation were prominent in all tissues, being more common and severe in lungs and livers. The livers exhibited acute hepatitis, necrosis and steatosis. Portal space inflammation was characterized by infiltration of T cells and macrophages. Both CD4+ and CD8+T cells were observed however most of them were CD8+, these cells were also found infiltrating parenchyma. Dengue antigen was visualized mostly in macrophages. In lungs alveolar hemorrhage, diffuse alveolar damage, hyaline membrane formation, type II pneumocyte hyperplasia and septal thickening was observed. The alveolar septum showed abundant mononuclear infiltration with macrophages and T cells, predominantly CD8+T cells. Macrophages were abundant also in the alveolar space, some of them with appearance of foamy histiocytes. Dengue antigen was observed in alveolar macrophages and type II pneumocytes. The lungs were the most affected organ, with alveolar diffuse hemorrhagic damage being a prominent pathological feature in fatal dengue. Abundant macrophages and moderate CD8+T cell infiltration were typical features in lung and liver lesions. Dengue antigen was present in alveolar macrophages and type II pneumocytes but rarely present in liver. These results highlight the participation of T cells and macrophages immune mediated responses in dengue induced organ pathology.

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CHRONIC MALNUTRITION DOES NOT IMPAIR THE MEMORY T CELL RESPONSE TO CRYPTOSPORIDIAL INFECTION IN INDIAN CHILDREN

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Approximately 48 % of children <5 years of age are stunted in India, and chronic malnutrition is believed to predispose to impaired immune responses and repeated infections. *Cryptosporidium* spp., an intracellular parasite is responsible for 3.1-7.6 million diarrheal episodes in children <2 years of age in India. Immune response to *Cryptosporidium* is primarily CD4 T cell based, with a poorly defined role for CD8 cells. This study aimed to assess the role of chronic malnutrition on memory T cell responses in children with cryptosporidial infections. In a birth cohort study of the natural history of cryptosporidium infection, infants from a semi-urban slum in Vellore, Southern India, were followed up from birth till 3 years of age, documenting all symptomatic and asymptomatic cryptosporidial infections. Morbidity and monthly anthropometric measurements were recorded, and children's growth classified using WHO guidelines. Peripheral blood mononuclear cells (PBMCs) were isolated at 3 years of age and were stimulated using *Cryptosporidium* oocyst lysate

and stained using florescent tagged antibodies to define the memory component of the CD4, CD8 T cells and markers to define Th1 (IFNγ), Th2(IL-4) and Th-17 (IL-17A) responses. Samples were acquired on a BD Aria III and analyzed. Thirty -four children who had symptomatic cryptosporidial infections were included for this preliminary analysis. Five children were persistently stunted (defined as having a HAZ score <-2SD at 6, 12, 18, 24, 30, 36 month time points) during the 3 year study period whereas 29 children were never stunted. Analysis of memory activated CD4 and CD8 cells expressing either the Th1, Th2 or Th17 phenotypes did not reveal any significant differences between the persistently stunted children compared to the never stunted children (p values not significant). The persistently stunted children had a robust IL-17A and IL-4 CD4 memory response, though they had a slightly lower IFNγ CD8 memory response as compared to the never stunted children. Chronically malnourished children with symptomatic cryptosporidial infections develop and maintain a good memory T cell response.

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GENETIC LINKS BETWEEN SYMPTOMATIC *ENTAMOEBA HISTOLYTICA* INFECTION AND INFLAMMATORY BOWEL DISEASE

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Diarrhea is the second leading cause of death for children globally, causing 760,000 deaths each year in children under the age of 5. Amoebic dysentery contributes significantly to this burden, especially in developing countries. To investigate possible genetic susceptibility to symptomatic *Entamoeba histolytica* (EH) infection in Bangladeshi infants, we conducted a genome-wide association study (GWAS) in two independent birth cohorts of symptomatic EH infection. Cases were defined as children with at least one diarrheal episode positive for EH through either PCR or ELISA within the first year of life, while controls were children without any episodes positive for EH in the same time frame (DBC: 65 cases, 309 controls; PROVIDE: 112 cases, 323 controls). The NIH Birth Cohort and the PROVIDE cohort were analyzed separately, using univariate logistic regression with an additive mode of inheritance. Results were meta-analyzed under a fixed-effects inverse variance weighting model. The top results were found within two neighboring genes on chromosome 10: *CUL2* (cullin 2) and *CREM* (cAMP responsive element modulator). A total of 4 SNPs (single nucleotide polymorphisms) met genome-wide significance (P<5E-08) in the joint analysis. The top SNP was rs2148483 with a P_{meta} of 6.48E-09, with additional risk allele at this locus conferred 2.3 times the odds of a symptomatic EH infection. This SNP is found within an intron of *CUL2* (cullin 2), which has previously been implicated as a susceptibility locus for Inflammatory Bowel Disease and Crohn's Disease. Despite neither individual analyses reaching significance due to their limited power, the meta-analysis of two independent studies shows genome-wide significant results in two genes previously implicated in IBD. These genetic associations reinforce the pathological similarities observed in gut inflammation between EH infection and IBD. Further research is needed to elucidate the underlying mechanisms for the proposed pleiotropy.

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RESPIRATORY TRACT CRYPTOSPORIDIOSIS IS COMMON IN HIV-SERONEGATIVE CHILDREN WITH INTESTINAL *CRYPTOSPORIDIUM* AND COUGH

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We studied respiratory tract cryptosporidiosis (RTC) in children aged 9-36 months presenting to the Acute Care Unit at Mulago Hospital (Kampala, Uganda) with a 2 different chief complaints: A) diarrhea, with cough (n=1023); and B) cough or pneumonia, with or without diarrhea (n=776). Children were screened for *Cryptosporidium* (CR) in stool using RT-PCR. Stool-positive children were selected for further diagnostic tests, including sputum induction. In addition, for every CR stool-positive child in Group B, a CR stool-negative child in this group was selected for further workup. Sputum samples were subjected to RT-PCR for *Cryptosporidium*, bacterial culture and sensitivity, auramine-smear for TB, and RT-PCR for RNA viruses (subject to adequate sample volume). Prevalence of intestinal cryptosporidiosis was 10.7% (89 *C. hominis*, 17 *C. parvum*, 3 *C. meleagridis* or mixed) and 4.8% (27 *C. hominis*, 9 *C. parvum*, 1 mixed) in Group A and B, respectively. Of the stool-positive children that underwent sputum induction, 28/85 (32.9%; Group A) and 8/29 (21.6%; Group B) had RTC. Strikingly, 2 stool-negative children in Group B, neither with diarrhea, also had RTC. All 38 children with RTC were HIV-seronegative. Preliminary data suggests *C. hominis* may have a greater propensity for infection at this site (e.g. RTC occurred in 37.1% of *C. hominis* enteric infections versus 16.7% of C. parvum enteric infections; Group A). Notably, RTC with *C. hominis* occurred even when C. parvum was the species detected in the intestinal tract. No bacterial pathogens were cultured from sputum of 8/28 children in Group A and 6/10 children in Group B with RTC. Results of respiratory virus testing were pending at the time of writing, however at least 3 children in Group B had no alternative explanation for their respiratory symptoms. Clinically, in Group A, children with RTC had significantly lower oxygen saturation SpO2 compared to those who were sputum-negative for *Cryptosporidium* (median 96% vs 98%, p=0.004). Enrolment continues with the aim of further elucidating the clinical and epidemiological significance of RTC.

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AZITHROMYCIN AND DOXYCYCLINE ATTENUATE *ACANTHAMOEBA* VIRULENCE IN A HUMAN CORNEAL TISSUE MODEL

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Amoebic keratitis (AK) is a potentially blinding eye infection caused by the parasite *Acanthamoeba*, a ubiquitous, free-living organism. This organism exhibits resistance to anti-acanthamoebal chemotherapy necessitating long treatment courses; despite this, treatment failures are common. *Acanthamoeba*e isolated from the environment and from the corneas of AK patients have been found to harbour bacterial endosymbionts belonging to Chlamydiales, Rickettsiales, and Legionellales. Previous studies demonstrated that a Chlamydia-like endosymbiont enhanced *Acanthamoeba* virulence *in vitro*, although it is unclear if this translates to a significant effect *in vivo*. We sought to elucidate the potential effect

of *Acanthamoeba*-endosymbiont co-infection in a human corneal tissue model representing clinical AK infection. Several environmental and clinical *Acanthamoeba* isolates from the ATCC were screened for endosymbionts by amplifying and sequencing bacterial 16S DNA. Each *Acanthamoeba* isolate was used to infect EpiCorneal cells, a 3D human corneal tissue model. EpiCorneal cells were then treated with azithromycin (AZT), doxycycline (DOX), or control media to determine if antibiotics targeting common classes of bacterial endosymbionts attenuated *Acanthamoeba* virulence as indicated by a decrease in observed cytopathic effect and inflammatory biomarker production. Infection of EpiCorneal cells with *A. castellanii* 50493 and A. polyphaga 50372 increased TNFα and IL-1 expression. This was attenuated with addition of AZT or DOX. IL-6 and Cu-Zn SOD expression also increased upon infection with either strain, yet attenuation with antimicrobials was only observed in *A. castellanii* 50493. Cytopathic effects on EpiCorneal cells were evident in the presence of *A. castellanii* 50493 and A. polyphaga 50372, and this was reduced with the addition of antimicrobials. Treatment of two *Acanthamoeba* strains with the endosymbiont-targeting antibiotics AZT and DOX, was shown to attenuate inflammatory cytokine production and reduce cytopathic effects. These drugs may present an alternative avenue of therapy in treatment of AK.

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CRYPTOSPORIDIOSIS IN A HUMANIZED MURINE INTESTINAL TRACT

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Cryptosporidiosis is as a major cause of persistent diarrhea worldwide. It has been recently cited as one of only 4 pathogens causing moderate to severe diarrhea in infants and young children in developing countries. Mouse models of enteric infections remain as powerful tools to study host response, susceptibility to disease and response to treatment. However in majority of the models, significant manipulation has been employed to attain infection, including genetic alteration, chemotherapy, irradiation, antibiotic exposure, germ-free condition, infection at neonatal period, or harsh dietary restriction, complicating potential relevance of findings to human disease. We tested the effect of *Cryptosporidium parvum* infection in a humanized murine model attained by gut flora conditioning using a child's fecal specimen. Dams were treated with an antibiotic cocktail and transplanted with human fecal material soon after conception. Control dams were treated with murine feces. Upon weaning, pups were fed with 2% protein diet and infected with *C. parvum* oocysts one week later. Pups were monitored for weight loss and stool specimens were collected up to 21 days post infection. Infected pups from dams transplanted with human feces lost weight, similar to infected pups from dams transplanted with murine feces. Interestingly, infected pups from dams transplanted with human feces shed the parasite longer than the control counterpart. Conditioning the murine intestinal tract with human flora *in utero* may increase susceptibility to cryptosporidial infection and result to prolonged shedding.

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IMPACT OF ENTERIC PARASITES ON INTESTINAL MICROBIOTA DIVERSITY AND METAGENOMIC CHANGES IN RURAL ARGENTINIAN AND ECUADORIAN CHILDREN

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Approximately 30% of children worldwide are infected with gastrointestinal (GI) parasites. Parasites can disrupt intestinal flora affecting nutritional status. We implemented a multi-parallel quantitative real-time PCR and whole genome sequencing analysis for bacterial microbiota and *Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus, Strongyloides stercoralis, Trichuris trichiura*, *Cryptosporidium, Entamoeba histolytica,* and *Giardia lamblia.* Stool samples were collected from 122 asymptomatic children (under 10 years old) from rural Argentina and Ecuador. Separate analyses were done by country for uninfected, *Giardia* only, *Giardia*/helminth co-infections, and helminth only groups. For *Giardia* only infected children, sequencing data showed a decrease in microbiota biodiversity compared to those uninfected that correlated with increasing *Giardia* burden (Spearman $r = -0.5491$, $p = 0.0244$). Clustering was statistically significant using Canonical Correspondence Analysis ordination and shannon alpha diversity (*Giardia* only 2.1; uninfected 2.7, $p < 0.05$). A non-significant increase in diversity was observed for helminth only infections (3.0) with a compensatory decrease in *Giardia*/helminth co-infections (2.3). In *Giardia* only infections, microbiome taxonomy shifted from *Firmicutes* towards increasing proportions of *Prevotella*, with degree of shift related to intensity of infection compared to uninfected (37.1 % versus 23.5%, $p = 0.037$). Abundance of *Prevotella* bacteria was decreased in the helminths only group, but increased for *Giardia*/ helminth co-infections (16.5% versus 38.3%, $p = 0.019$). Metagenomic analysis of the microbiota showed a significant increase in genes required for anaerobic activity among the *Giardia* only group compared to all children without *Giardia* ($p = 0.012$). Our data provides possible evidence for an affect of *Giardia* infections on the intestinal environment allowing permissive growth of anaerobic bacteria such as *Prevotella* and a decrease in microbiota diversity. Future work will explore the contribution of such changes to growth delays in parasite-infected children.

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THE GENOME SEQUENCE OF ANTHROPONOTIC *CRYPTOSPORIDIUM PARVUM*

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Cryptosporidiosis is a life-threatening disease in immune-compromised individuals worldwide, and a major cause of diarrhea-induced death of young children in developing countries. The large Global Enteric Multicenter Study (GEMS) revealed that cryptosporidiosis infections in the developing world are caused primarily by *Cryptosporidium hominis,* followed by *C parvum.* Recent studies revealed that the majority of *C. parvum* infections are due to anthroponotic genotypes. Specifically, it has been observed that, in developing countries, the transmission of *C. parvum* occurs typically from human to human, while zoonotic infections seem to be dominant in developed countries. Until now, *C. parvum* genomic resources have been based on zoonotic isolates, particularly the isolate IOWA II, the source of the reference genome for the species,

and the first *Cryptosporidium* genome to be published. Here, we re-sequenced the genome of the anthroponotic *C. parvum* isolate TU114 using Pacific Biosciences technology, and generated a comprehensive, genome assembly with few sequencing gaps. Comparison between this genome and those of zoonotic isolates will enable a more comprehensive understanding of transmission mechanisms of cryptosporidiosis in humans and animals, and aid vaccine development research against anthroponotic subtypes of *C. parvum*.

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POPULATION GENOMICS OF *PLASMODIUM FALCIPARUM* TO INFORM THE DESIGN AND EFFICACY OF WHOLE ORGANISM MALARIA VACCINES

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The Sanaria® PfSPZ Vaccine is a radiation-attenuated whole-organism vaccine based on the sporozoite stage of *Plasmodium falciparum* (Pf). The vaccine isolate NF54 (West Africa) is the isolate from which the Pf reference 3D7 was cloned. While vaccine efficacy after immunization with moderate doses of PfSPZ is high and similar against controlled human malaria infection (CHMI) with homologous (3D7) and heterologous (7G8 from Brazil) clones, at lower doses protection is higher against CHMI with 3D7 compared to 7G8. Furthermore, at lower doses protection may be lower in the field than against CHMI. These results suggest that while broad efficacy can be achieved by increasing the dose, it may be achieved more efficiently and at lower doses by including additional Pf strains in the vaccine. This goal requires rigorous characterization of NF54 and comparisons to Pf populations in malaria endemic regions so that complementary vaccine strains can be selected. To this end, NF54 was sequenced with PacBio and Illumina platforms resulting in a high-quality assembly and SNP calls. The resulting assembly consists of 24 contigs with a cumulative length of 23.28 Mbp, similar to the 3D7 reference genome (23.26 Mbp). Of the 5,542 genes in the 3D7 genome, all but 15 map completely and accurately to the NF54 genome. Both SNP calls and the assembly indicate that NF54 is very similar to 3D7, with <3K SNP differences between them. We then sequenced and assembled the genomes of 3 additional Pf clones proposed as vaccine constituents: 7G8, NF135.C10 (Cambodia), and NF166 (Guinea). Along with NF54, these were then compared to several hundred Pf clinical isolates from around the globe using whole-genome sequence data. Principle coordinate and admixture analyses based on SNP calls show that cloned and clinical isolates cluster based on geographic origins. Focusing on pre-erythrocytic antigens, we investigate allele and epitope frequency of the four proposed vaccine strains among global Pf populations to inform vaccine design and to interpret the outcome of upcoming vaccine trials.

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GLOBAL LANDSCAPE OF MOLECULAR NETWORKS FOR MALARIA PATHOLOGY REVEALED BY INTEGRATIVE MULTI-OMICS ANALYSIS USING NON-HUMAN PRIMATE ANIMAL MODEL

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Non-Human Primate (NHP) animal models infected with *Plasmodium* pathogens can provide insights into mechanisms and help discover biomarkers for human malarial disease conditions. We adopted a systems biology strategy to comprehensively measure host physiological response to *P. cynomolgi* and *P. coatneyi* infection longitudinally at various molecular layers, including transcriptomics, metabolomics, lipidomics, immunological profiling, proteomics and clinical traits in Peripheral Blood (PB) and Bone Marrow (BM). We developed a novel network biology analysis method, CLR-Directed Bayesian Network Analysis (CDBNA), to integrate Omics data for interrogating differential and common multi-omics network modules perturbed by *P. cynomolgi* and *P. coatneyi* and for deciphering network dynamics in the course of infection. This method integrates the advantages of both information theory-based methods (Context Likelihood Relatedness, CLR) and Bayesian Network Analysis (BNA): CLR empowering genome-scale network reconstruction and nonlinear relationship discovery, with BNA enabling high-resolution focused study of CLR-identified sub-networks. We first applied CLR to integrate Omics data from PB and BM infected with *P. coatneyi* or *P. cynomolgi*, providing a low-resolution overview on genome-scale networks. Then, we applied BNA to derive conditional dependencies between nodes in the network for focused pathways. We illustrate the power of CDBNA by identifying roles of INF-gamma related pathway in malaria. An atlas of species-specific (between *P.coatneyi* and *P. cynomolgi*) and tissue-specific (between PB and BM) network modules of malaria were identified, which highlights tissue-specific mechanisms as well as convergent and divergent mechanisms between *P. coatneyi* and *P. cynomolgi* affecting host network. We illustrate the power of integrative multi-omics analysis by identifying roles of key processes related to immune memory and inflammation in malaria. This global multi-omics map of malaria pathology provides a scaffold for disease mechanism study and potential novel therapeutic targets for prevention and treatment of malaria.

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PIGGYBAC MUTAGENESIS SCREENING OF THOUSANDS OF *PLASMODIUM FALCIPARUM* GENES REVEALS WHAT A MALARIA PARASITE CAN'T LIVE WITHOUT

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Malaria remains an important global health problem and emerging resistance to frontline drugs may reverse recent progress. More robust genomic tools are needed to accelerate progress to identify and validate essential candidate targets to develop the most effective new antimalarial therapies. The efficiency of the *piggyBac* transposon system for insertion within a wide range of genomes and its propensity for random selection of its highly preferred TTAA tetranucleotide insertion site has led to its growing use for functional genomics studies in multicellular eukaryotes. Using Quantitative Insertion-site Sequencing (QIseq), our newly developed next-gen sequencing tool to identify *piggyBac* insertion sites, a high density of insertions revealed a dramatically skewed distribution relative to the frequency of expected genomic *piggyBac* TTAA insertion sites in the

Plasmodium falciparum genome. Through analysis of >5000 new unique insertions generated via a method that primarily produces single-insertion mutant parasites, we can discern genes and pathways essential and dispensable for intraerythrocytic growth of an NF54 clone grown in routine *in vitro* culture. Most importantly, regions significantly void of insertions are calculated to represent regions of genes with essential functions in blood-stage development whereas genes with insertions are considered to be dispensable. Over 1500 insertions are estimated to be lethal in the mutagenesis screen, which was validated by comparison with results for *P. berghei* knock out screens. We will present results from our study that includes analysis to identify approximately 500 high-confidence essential *P. falciparum* genes, representing crucial biological processes associated with core metabolic functions during intraerythrocytic growth. Our results establish *piggyBac* mutagenesis as an effective genetic tool to distinguish essential and dispensable genes that will help accelerate development of new antimalarial therapies.

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WHOLE GENOME SEQUENCING OF *PLASMODIUM FALCIPARUM* MALARIA PARASITES FROM DRIED BLOOD SPOTS: GATEWAY TO HIGH-RESOLUTION GENOMIC SURVEILLANCE

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Plasmodium falciparum malaria is hyperendemic and seasonal in the Kassena-Nankana Districts (KNDs) of the Upper East Region of Ghana, causing significant morbidity and mortality. Medications used for disease control include sulfadoxine-pyrimethamine (SP) prophylaxis during pregnancy, and artemisinin-based combination therapies (ACT). Monitoring the parasite evolutionary response to such measures can inform public health, e.g. by tracking genetic markers that confer or predispose to SP and ACT resistance. But genome-wide epidemiology is limited by technical and logistical difficulties in obtaining sequenceable material from clinical samples. Here, we describe a method for generating *P. falciparum* whole genome sequences from dried blood spots (DBS), collected from finger-pricks (~30µl of blood) in resource-limited rural settings in the KNDs. Using selective whole genome amplification (sWGA), parasite DNA with high (>95%) host contamination was selectively amplified using oligo nucleotides that preferentially bind to the parasite genome. We analysed *P. falciparum* genomes from 156 patients with clinical malaria, including 120 paired DBS and leucocyte-depleted venous blood (VB) samples for head-to-head comparison of sWGA vs genomic DNA. More than 90% of DBS samples produced parasite-enriched DNA with >95% of the core *P. falciparum* genome covered with ≥5x sequence reads, allowing drug resistance loci such as *crt*, *dhfr*, *dhps*, *mdr1*, and *kelch13* to be genotyped. Concordance across ~1 million single nucleotide polymorphisms between VB and DBS samples was >94%, despite there being 50x less blood and no leucodepletion in the DBS samples. Quality sequence data was obtained from DBS down to parasitaemias of 0.03% (~40 parasites per 200 white blood cells). Thus, sWGA makes large-scale, high-resolution genomic epidemiology possible, as DBS collection is better tolerated by most patients and more suitable for remote, resource-limited settings than VB. Genomic data can then inform proactive public health strategies to combat evolving pathogens.

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CULTURE-ADAPTATION OF MALARIA PARASITES IS ASSOCIATED WITH NONSENSE MUTATIONS IN SPECIFIC GENES

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Substantial developments in understanding malaria parasite biology have been derived from studies of *Plasmodium falciparum* cultured *in vitro*. However, culture-adapted laboratory strains may differ from parasites in clinical isolates. To investigate the genetic basis of parasite culture adaptation, 50 new Gambian clinical isolates were introduced to standard *in vitro* culture conditions. Fourteen (28%) of the isolates were successfully grown for at least 48 days, and some were subsequently continued for longer periods. Six of these contained unmixed haploid genotypes at day 0, and were chosen for genome sequence analysis of samples taken during the culture period. In three of the isolates new single nucleotide polymorphism (SNP) variants emerged to frequencies of above 20% during culture, becoming the majority sequences in two isolates. Out of five novel SNPs, four were nonsense mutations resulting in stop codons. Three of these were in the same gene encoding an ApiAP2 transcription factor, with each isolate having a different nonsense mutation, and one was in a serine/threonine protein kinase gene (*SRPK1*). These results prompted survey for nonsense SNP alleles in genomes of eight widely cultured laboratory-adapted parasite strains, revealing four different nonsense mutations in other *ApiAP2* genes, one of which has been previously reported. Remarkably, five of the eight laboratory strains each had a different nonsense mutation in the rap guanine nucleotide exchange factor (*Epac)* gene. A global database of uncultured clinical samples revealed that nonsense mutations were very rare overall, and never found in *Epac*, *SRPK1*, or ApiAP2 genes. In conclusion, loss-of-function mutations in a small number of specific genes are associated with *P. falciparum* growth in culture.

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TRANSCRIPTIONAL AND PROTEOMIC CATEGORIZATION OF THE ETIOLOGY OF PNEUMONIA SYNDROME IN PEDIATRIC PATIENTS IN MALARIA ENDEMIC AREAS

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Pneumonia and malaria are common causes of pediatric respiratory distress in tropical settings. Bacterial pneumonia in particular is a major contributor to morbidity and mortality among children, principally due to delay in antibiotic therapy. Proper clinical treatment requires an actionable and accurate diagnosis. A rapid test that could distinguish between bacterial, malarial, and viral infections would have great clinical utility in directed life-saving antibiotic therapy. We first performed RNA-Seq and analyzed the transcriptomes of 68 pediatric patients with well-characterized clinical phenotype to identify transcriptional features associated with each disease class. Next, we refined those features and used them to create predictive models using elastic net and support vector

machine algorithms. Finally, we validated those models on an independent test set of 37 patients. We developed 4 RNA-based models based on gene expression to distinguish between bacterial, malarial, and viral infections. Support vector machine models fit the training set perfectly utilizing 600 marker genes, suggesting overfitting; elastic net models by contrast had a more uniform performance between train and test sets while utilizing significantly fewer genes. In a second approach, we performed Luminex-based and aptamer-based proteomic analysis and characterized the peripheral blood protein response of 161 pediatric patients from the same population. The pipeline for analysis initially included partial least squares, PAM (prediction analysis for microarrays, a Tibshirani method), random forests, and elastic nets (with genetic algorithms). This study demonstrates that human transcriptional features and proteomic signatures in patients with infectious disease diagnoses recapitulate the underlying biology and provide models for predicting diagnosis. We have identified sets of genes and specific groups of proteins that are expressed in pediatric patients with pneumonia syndrome attributable to different infections and requiring therapeutic interventions. These may provide the foundation for a clinical point of care diagnostic.

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RISK FACTORS FOR SECONDARY HOUSEHOLD TRANSMISSION OF INFLUENZA VIRUS AH3N2 IN THE PERUVIAN NORTHERN COAST

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Influenza virus is associated with 250,000 to 500,000 deaths worldwide and household transmission accounts for approximately one third of all cases. We evaluated risk factors associated with secondary household transmission of influenza virus AH3N2 in a rural community in the northern coast of Peru through a prospective population-based cohort study of influenza-like illness (ILI). We made household visits three times per week to detect cases of ILI, collect nasopharyngeal samples from cases and testing them by RT-PCR. We identified index cases as the first ILI case reported in a household with laboratory-confirmed influenza AH3N2. People residing in the same household as the index case were considered household contacts. Secondary cases were household contacts who developed laboratory-confirmed influenza AH3N2 ILI within seven days after the index case. The household secondary attack rate (SAR) was calculated and generalized linear models were fitted to identify the potential risk factors for transmission, accounting for household clustering, population size, number of primary cases, and other relevant descriptors. The SAR was 6.9% [CI 95% 4.3-10.2] with 22 secondary cases among 321 household contacts. In multiple regression analysis, secondary risk increased 3.6 times in household contacts <5 years old versus those older [Adjusted Prevalence Ratio(PR)=3.6, 95%CI: 1.4-9.3] and risk was increased 4.7 times to siblings of the index case compared to other contacts [Adjusted PR=4.7, 95%CI: 1.4-15.4]. Household transmission of influenza AH3N2 virus was frequent in this cohort, especially to young siblings, who are likely to have especially close contact with the index case. Seasonal vaccinations of children as well as non-pharmaceutical interventions may mitigate this risk. More studies with a better proxy of close contact between household members are recommended.

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DISTRICT TRENDS IN UNDER-FIVE PNEUMONIA MORTALITY IN MALAWI, 2000-2014

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In Malawi, under-5 mortality fell from 247 deaths per 1000 live births in 1990 to 71 in 2013, meeting the target set by Millennium Development Goal 4. This decline was due, in part, to a reduction in pneumonia mortality, which fell from 27.5 under-five pneumonia deaths per 1000 live births to 10.1 from 2000 to 2014. The study objective is to document variability in the decline in under-five pneumonia mortality in Malawi from 2000 to 2014 across 28 districts and describe the contribution of health interventions to this decline. We used the Lives Saved Tool (LiST, version 5.41, Beta 6) to estimate the impact of change in intervention coverage on under-five pneumonia mortality by district from 2000 to 2014. Estimates of intervention coverage were drawn from the Demographic Health Survey (2000, 2004, 2010) and Multiple Indicator Cluster Survey (2006, 2014), and interpolated for years when survey data were unavailable. Populations and cause of death data came from World Population Prospects 2015. We compared the contribution of preventive (breastfeeding, vaccination, nutrition) and curative (antibiotics) interventions on district change in pneumonia mortality. Results for two districts, Blantyre and Kasungu, with different baseline care-seeking, are presented as an illustrative example. The proportion of deaths attributed to pneumonia among children 1-59 months decreased from 17.7% to 15.5% in Blantyre from 2000 to 2014 and from 20.7% to 11.6% in Kasungu. Blantyre had higher care-seeking for pneumonia, 36.2%, while Kasungu was lower at 13.1% in 2000. In 2014, 86.5% of children in Kasungu received 3 doses of PCV while 74.9% did in Blantyre. Vaccination averted >2800 additional pneumonia deaths in Blantyre from 2001 to 2014, accounting for 75% of deaths averted. Vaccination averted 34.2% of additional pneumonia deaths in Kasungu and increase in care-seeking prevented >3000 (30.7%) of pneumonia deaths. Health interventions were scaled up at different rates across districts, which resulted in variations in change in pneumonia mortality over time. Planners should disaggregate data to ensure all districts are served by interventions.

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EPIDEMIOLOGY OF INFLUENZA AMONG SEVERE ACUTE RESPIRATORY INFECTIONS — DAMANHOUR, EGYPT, 2009-2015

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Influenza infections cause substantial morbidity and mortality. We sought to estimate the incidence and describe the characteristics of influenza infections among severe acute respiratory infections (SARI) cases in Damanhour, Egypt. We analyzed surveillance data of SARI cases from 3 public hospitals from June 2009-December 2015. SARI was defined as history of fever or measured temperature ≥38°C, cough with onset in the last 10 days, and requiring hospitalization. Polymerase chain reaction (PCR) of naso/oropharyngeal specimens were tested for influenza A and B viruses, adenovirus, parainfluenza virus, respiratory syncytial virus, human metapneumovirus, and coronavirus. Data from a 2012 healthcare utilization survey were used to estimate incidence. Among 10,431 SARI cases, 9,992 (95.8%) had PCR testing; of these, 3,664 (36.7%) were

positive for one of the tested pathogens and 1,569 (15.7%) for influenza. Among influenza infections, the proportion positive was 57.5% (n=902) for influenza A, 35.9% (n=563) for influenza B, and 6.6% (n=104) for co-infection with other tested viruses. Frequencies for influenza A subtypes were 58.1% (n=524) for seasonal H1N1, 48.9% (n=441) for H3, and 0.8% (n=7) for H5. Overall incidence of influenza infection was 96.9 per 100,000 person-years. The highest proportion of influenza infections occurred during October-February (769/3,409, 22.6 %) compared to other months (206/4,488, 4.6%), (p<0.001). Influenza A infections were 4.9 times more likely between October-November compared to other months. Mean duration of symptoms was 5.17+2.3 days and hospitalization was 4.0+3.1 days; 28 (1.8%) patients were admitted to intensive care for a mean duration of 5.9+5.9 days. Patients with influenza A infection were 2.2 times more likely to be admitted to intensive care compared to patients with influenza B infection. Three cases died, two of whom had influenza A infection. Influenza infection peaked during winter primarily from influenza A seasonal H1N1 and H3. Infection with influenza A was associated with more severe disease and higher mortality. Routine vaccination is essential to decrease influenza burden.

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QUALITY OF CASE MANAGEMENT OF PNEUMONIA AND DIARRHEA IN CHILDREN AGED <5 YEARS: RESULTS FROM A HEALTH FACILITY SURVEY IN SOUTHERN MALAWI IN JANUARY-MARCH 2015

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Globally, pneumonia and diarrhea are leading causes of child deaths. Integrated Management of Childhood Illness (IMCI) is a widely adopted approach to manage these and other childhood illnesses in resource-poor settings, but studies show that many children are inappropriately treated. The objectives of this analysis are to describe case management in Malawi, a malaria-endemic country, in children aged 2-59 months for pneumonia per IMCI and aged <5 years for diarrhea, and to determine factors associated with case management quality. During January-March 2015, we conducted a cross-sectional survey of 95 health facilities (HFs) in southern Malawi using patient exit interviews, healthcare worker (HCW) interviews, and HF assessments. Results were weighted and logistic regression models examined patient, HCW, and HF factors associated with pneumonia and diarrhea case-management quality, using local IMCI guidelines as the gold standard. Of 694 children aged 2-59 months, 132 (19.4%) met IMCI criteria for uncomplicated pneumonia. Of those, HCWs gave correct treatment (cotrimoxazole, amoxicillin, or erythromycin) to 90 (62.8%) and diagnosed pneumonia in 24 (15.1%). Of 724 children aged <5 years, 222 (27.2%) met criteria for uncomplicated diarrhea. Of the 222, HCWs diagnosed 135 (63.7%) and correctly treated 94 (38.2%) with oral rehydration solution (ORS) with or without zinc. Multivariable analyses showed that HCW ascertainment of cough or difficult breathing was associated with correct pneumonia treatment (odds ratio [OR]: 3.1; 95% confidence interval [CI]: 1.01-9.8). Children were more likely to receive correct diarrhea treatment if female (OR: 2.7; 95% CI: 1.3-5.6), if HCWs diagnosed diarrhea (OR: 6.3; 95% CI: 3.1-12.6), or at facilities with ORS in stock (OR: 4.6; 95% CI: 1.8-11.4). Malaria diagnosis was negatively associated with correct management of both pneumonia (OR: 0.4; 95% CI: 0.2-0.8) and diarrhea (OR: 0.3; 95% CI: 0.1-0.7). To improve quality of care, HCWs should be encouraged to solicit patient symptoms systematically and consider alternative common illnesses in children even when malaria is suspected.

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NEGLECTED TROPICAL POPULATIONS: THE BURDEN OF INFLUENZA IN THE ELDERLY, THAILAND

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As the global population ages, infectious diseases of older adults may increase in importance. Influenza virus infections disproportionately affect the very young and the very old, but little attention is given to influenza in the elderly in tropical countries, where influenza was not recognized as an important cause of illness until recently. We used systematic, random sampling to recruit a population-based cohort of persons aged ≥65 years in Nakhon Phanom province, northeastern Thailand, to measure the burden of influenza and the effectiveness of the influenza vaccine to prevent influenza-associated acute respiratory infections (ARI). Participants were contacted weekly to identify those with ARI, and samples were taken by participants at home by self-swabbing their anterior nares. Nasal swabs were tested for influenza viruses using PCR. In May 2015, we enrolled 3,219 elderly persons into the study. Influenza vaccination was offered to the elderly population at large in Nakhon Phanom but was not linked to study enrollment. The cohort contributed 2,512 person-years of observation through the end of March 2016. The median age at enrollment was 71 years (interquartile range, 68-76), 59% of the participants were female and 52% received an influenza vaccine in 2015. There were 1029 ARI cases, with 1013 (98%) samples tested; 42 (4%) samples were positive for influenza, of which 31 (74%) were influenza A/H3N2, 7 (17%) were influenza A/H1N1pdm09 and 4 (10%) were influenza B. Most (83%) of the influenza infections were detected between June and November 2015 (the influenza season), and the incidence of influenza-associated ARI during the influenza season was 3 per 100 p-y (95% confidence interval, 2-4). In this population of Thai elderly with a high influenza vaccination rate, the incidence of influenza-associated ARI was moderate and most illness was associated with influenza A/H3N2. Further analysis will be conducted to determine the burden of hospitalization caused by influenza and the effectiveness of the influenza vaccine in preventing illness in this cohort. This information may be useful to prioritize limited vaccine resources in Thailand.

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ROLE OF NASOPHARYNGEAL PNEUMOCOCCAL DENSITY IN THE EVOLUTION OF ACUTE RESPIRATORY ILLNESSES IN YOUNG PERUVIAN CHILDREN

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Streptococcus pneumoniae commonly colonizes the nasopharynx of young children, with colonization a critical initial step in the development of pneumococcal disease. We analyzed surveillance data from a prospective cohort of young children to examine pneumococcal nasopharyngeal density patterns surrounding acute respiratory illness (ARI) in a rural community setting of the Peruvian highlands. We assessed ARI with weekly household visits and collection of nasal swabs for viral detection. We also collected monthly nasopharyngeal samples to quantify pneumococcal colonization. We defined pre- and post-ARI periods as the

14 days before and after an ARI, and compared pneumococcal densities among samples collected during non-ARI, pre-ARI, ARI and post-ARI periods. Nasopharyngeal samples (n=3,579) from 837 children (median age 1.39 years) were included; 69% of samples had pneumococcal colonization. Relative to non-ARI, median pneumococcal density increased during pre-ARI periods and peaked during ARI. In secondary analyses of samples collected during ARI, both the presence of rhinovirus and persistent detection of colonization (with a new or previously detected serotype) were associated with higher pneumococcal density. These data suggest that nasopharyngeal pneumococcal density is dynamic surrounding episodes of ARI.

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DEVELOPMENT AND CLINICAL PERFORMANCE OF A HIGH THROUGHPUT LOOP-MEDIATED ISOTHERMAL AMPLIFICATION SYSTEM FOR THE DETECTION OF MALARIA

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As malaria prevalence declines in many parts of the world, the accurate and efficient detection of very low density malaria parasite infections is crucial for enabling rapid treatment and interruption of disease transmission. This is increasingly important as more endemic countries move towards elimination with community surveillance and treatment of sub-patent and asymptomatic infections. Common detection methods, based on microscopy and rapid diagnostic tests (RDTs), allow quick and accurate detection but are unable to identify most blood-stage infections below 50 parasites/µl. Nucleic acid amplification techniques (NAATs), such as PCR, are capable of detecting trace amounts of parasite DNA but are costly and complex to establish and maintain in endemic settings. Loop mediated isothermal amplification (LAMP) is a NAAT where amplification occurs in one step under isothermal conditions and is commercially available. The LAMP malaria kit (Loopamp® from Eiken Chemical Co.) was demonstrated to have excellent diagnostic performance in simple laboratories of various endemic countries. However its sample processing and overall throughput was low, limiting the deployment of this technique for large scale screening campaigns. In this study, we evaluate the clinical performance of a newly developed high throughput (HTP) sample processing system to be used in conjunction with the malaria LAMP kit. This system is based on a 96 well format that significantly reduces the need for individual sample pipetting by the use of a simple vacuum system and is compatible with the use of fresh blood and dried blood spots. The turnaround time from sample processing to reading results for 96 samples is less than 2 hours. Our results show that this relatively simple high throughput DNA extraction method for LAMP is capable of detecting low density infections down to 1p/µl, with similar diagnostic accuracy to the gold standard PCR. These characteristics show the HTP-LAMP system is a robust and highly sensitive diagnostic test, with the potential to allow large scale sensitive population screening in the context of malaria elimination campaigns.

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DEVELOPMENT OF HIGHLY ACCURATE QPCR ASSAYS FOR QUANTIFICATION OF SUB-MICROSCOPIC MALARIA PARASITES IN ASYMPTOMATIC POPULATION

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In malaria endemic areas, most infections with the parasite in older children and adults are asymptomatic, and are often characterized by sub-microscopic parasitemia. These often accounts for the majority of the total prevalence of infection as opposed to clinical disease. Asymptomatic malaria inadvertently contributes to malaria transmission due to the long duration of infection and high incidences of gametocyte carriage in both high and low transmission areas. Therefore, the need for highly sensitive malaria diagnostic tools cannot be over emphasized. Here, we describe the development and validation of sensitive plasmodium real time qPCR assays for the detection of low-level malaria parasites in blood. We will also report the prevalence of malaria parasitemia as determined by the assays. Using 700 samples collected in a HIV/malaria co-infection study in a malaria endemic region in western Kenya, the presence of any species of malaria was first determined using an improved assay based on genus-conserved sequences of the *Plasmodium* 18S ribosomal gene. All infections with ≥1 parasite per 50ul sample were detectable by our assay. Second, if positive for malaria, a panel of highly species-specific qPCR assays were developed to determine the presence and quantity of mixed species infections including *P. falciparum, P. ovale* and *P. malariae*. Finally, due to the importance of asymptomatic parasitaemia in malaria transmission, the presence and quantity of gametocytes was determined using stage-specific reverse-transcriptase qPCR. We will report the assay parameters including the limit of detection (LOD) and limit of quantification (LOQ), the linearity of the standard curves over a range of plasmid concentrations, coefficient of variation scores and the PCR efficiency. Overall, these results show sensitive quantitative PCR assays that can be used on samples from malaria endemic areas where high prevalence of sub-microscopic parasitemia have been reported. Such data can be used to inform public health measures aimed at reducing malaria transmission in communities.

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VALIDATION OF ULTRASENSITIVE DETECTION OF ASYMPTOMATIC MALARIA USING DRIED BLOOD SPOTS

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Southeast Asian countries are committed to eliminating artemisinin-resistant *Plasmodium falciparum* malaria in the region by 2030. Recently, ultrasensitive PCR techniques capable of detecting ultralow parasitemias have been used to select target populations for mass drug administration to eliminate malaria infections in asymptomatic carriers who may represent a transmission reservoir. However, these techniques require the use of either venous blood or preserved capillary blood. An ultrasensitive test for malaria infection that can be done using dried blood spots (DBS) would have potential to be scaled up for widespread surveillance. Here we report an optimized method for the highly sensitive detection of *P. falciparum* and *P. vivax* infections using DBS that is both high-throughput and cost-effective, with a similar sensitivity to methods based on whole blood. Laboratory experiments demonstrate a lower limit of detection (LoD) of 20 parasites/mL for DBS collected on Whatman 3MM papers and of 23 parasites/mL for Whatman 903 Protein Saver cards, about 5,000-fold more

sensitive than rapid diagnostic tests and similar to the 16-22 parasites/mL reported for other non-DBS ultrasensitive methods. We validated the sensitivity of the method in two field studies in Myanmar that took place during the wet and dry season. Nearly identical prevalence estimates of subclinical malaria were obtained from DBS samples as with preserved capillary blood samples, as well as with an independent ultrasensitive method using venous blood samples. These data validate the utility of DBS for use in asymptomatic surveillance studies. We are currently using DBS-based ultrasensitive PCR for large-scale surveillance studies across Myanmar in support of malaria elimination.

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DETECTION OF *PLASMODIUM FALCIPARUM* DNA IN SALIVA SAMPLES STORED AT ROOM TEMPERATURE : POTENTIAL FOR A NON-INVASIVE SALIVA-BASED DIAGNOSIS OF MALARIA

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Current malaria diagnostic methods are invasive, requiring blood collection with inherent risk of contracting blood-borne pathogens, pain and poor compliance when repeated sampling is required. On the other hand, the use of saliva, which is a minimally invasive sample for the diagnosis of malaria, has not been widely evaluated. We aim to evaluate the diagnostic test performance of saliva collected and stored at room temperature using the OMNIgene®•ORAL kit, in the diagnosis of malaria. Concurrent blood and saliva samples were collected from 224 febrile patients in Cameroon. Saliva samples were collected in the OMNIgene•ORAL (OM-501) kit and stored at room temperature. Detection of *Plasmodium falciparum* (Pf) DNA was based on amplification of the multicopy 18s rRNA gene using nested-polymerase chain reaction (nPCR). Light microscopy was used to detect Pf blood-stage parasites. Microscopy, nPCR-saliva and nPCR-blood based prevalence of malaria was 22%, 29% and 35%, respectively. When microscopy was used as gold standard, the sensitivity of nPCR-saliva and nPCR-blood in detecting Pf was 91% and 100%, respectively; however, the specificity was 92% and 87%, respectively. When nPCR-blood was used as gold standard, the sensitivity of nPCR-saliva and microscopy was 80% and 68% respectively; whereas the specificity was 99% and 100%, respectively. Nested PCR-Saliva had a "very good" agreement with both microscopy (kappa value 0.8) and blood PCR (kappa value 0.8). Nested PCR-Saliva detected 16 sub-microscopic malaria infections whereas 30 sub-microscopic infections were identified by nPCR-blood. At parasitemia >10,000 parasites/ml of blood, the sensitivity of nPCR-saliva was 100%. Saliva can be used, as an alternative non-invasive sample for the diagnosis of malaria and the OM-501kit is effective at transporting and preserving malaria parasite DNA in saliva at room temperature.

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STIMULATING THE MARKET FOR MALARIA RDTS: NOVEL INSIGHTS FROM REAL-WORLD PROGRAMMING FOCUSED ON PRIVATE SECTOR SERVICE DELIVERY AND MARKET DEVELOPMENT

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Almost 8 out of every 10 suspected malaria patients received a diagnostic test in the public sector in 2014. Estimates of the level of private sector testing are much lower. This is a public health concern given that a significant proportion of patients (estimated at 40%) in many malaria endemic countries seek care for febrile illnesses from private sector providers. Malaria rapid diagnostic tests (RDTs) are considered a viable option for resource-constrained settings. The success of national diagnostic strategies will depend on increased availability and appropriate use of quality-assured RDTs in the private sector. Recent small-scale studies have introduced RDTs into the private sector to estimate their impact on fever case management. Given their controlled settings, these studies offer limited insight into the real world issues of supply chain reliability, RDT affordability, marketing and demand creation among providers and clients, acceptability of test results to providers and clients, post-market quality control of RDTs, and interface with country diagnostic policy and guidance. In response to these knowledge gaps, PSI in collaboration with partners (Malaria Consortium, FIND, and Johns Hopkins School of Public Health) implemented a three-year initiative funded by UNITAID to stimulate a private sector market for RDTs in five sub-Saharan African countries: Kenya, Tanzania, Madagascar, Uganda and Nigeria. Designed as an operations research project, the consortium successfully completed 44 studies across the 5 countries. This presentation will offer an overview of the project design, findings and lessons learned, situating them in relation to results from other interventions in this area. In doing so, the presentation will enable participants to understand to 1) the important role the private sector can play in fever case management in Africa; 2) key market shortcomings, quality concerns and policy challenges related to the scale-up RDTs in the private sector in Africa; and 3) how the new WHO Roadmap for public-private engagement (expected release date of September 2016) can help address these challenges.

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SINGLE CELL FUNCTIONAL ARTEMISININ RESISTANCE IN CLINICAL STRAINS OF *PLASMODIUM FALCIPARUM* MALARIA

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Emergence and spread of artemisinin resistance raises risk of wiping out recent gains achieved in reducing worldwide malaria burden and threatens future malaria control and elimination on a global level. *Plasmodium falciparum* Kelch13 (PfKelch13) and the ring stage survival assay (RSA) respectively provide important tools to screen for and validate the spread of artemisinin resistance. But they fail to detect Kelch-independent mechanisms and a large proportion of clinical strains cannot be adapted for RSA, hampering comprehensive mapping and understanding the global biology of artemisinin resistance, in particular its heterogeneity in individual parasites and clinical infection. Our prior studies have shown that artemisinins target *P. falciparum* phosphatidylinositol-3-kinase (PfPI3K), which binds Kelch13. Kelch13 mutations of artemisinin resistance decrease kinase binding to elevate the lipid product phosphatidylinositol-3-phosphate (PI3P), as reported previously. PI3P increase is predictive of resistance across clinical and engineered laboratory parasites even in absence of PfKelch13 mutations, suggesting that quantitative detection of PI3P in individual parasites may provide a powerful index of resistance. However separation of PI3P from phosphatidylinositol-4-P (PI4P) is challenging since both lipids have identical mass and charge. Here we report on functional assays that quantitatively detect PI3P as well as downstream pathways and distinguish them from PI4P in single *P.falciparum* parasites. Evidence will be presented for rapid measurements of artemisinin resistance applicable to clinical strains eliminating need for culture adaptation and providing powerful mechanistic portals for both the biology of resistance and its heterogeneity in infection.

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DELETIONS OF PFHRP2 AND PFHRP3 IN RDT-NEGATIVE *PLASMODIUM FALCIPARUM* ISOLATES FROM UGANDA

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Malaria rapid diagnostic tests (RDTs) play a key role in malaria case management. The most widely-used RDT identifies *Plasmodium falciparum* based on immunochromatographic recognition of PFHRP2. Deletion of pfhrp2 was reported to be common in *P. falciparum* isolates from Peru and Ghana, and uncommon in isolates from Mali, Senegal, and India. We investigated the presence of deletions in pfhrp2 and the homologous gene pfhrp3 in samples collected from cross-sectional surveys conducted in 3 regions of Uganda in 2012-13. The surveys included annual blood smears and HRP2-based RDTs (SD BIOLINE) in children and adults from randomly identified households. Of 1,493 samples with positive blood smears, 96 were RDT negative and selected for further investigation. DNA was extracted from dried blood samples using Chelex-100. Analysis included amplification of subunit ribosomal DNA for *P. falciparum*-specific sequences, amplification of pfhrp2 and pfhrp3 by nested PCR followed by electrophoresis of PCR products to identify gene deletions, and amplification and electrophoresis of polymorphic regions of msp2 to assess complexity of infection. Of the 96 samples, *P. falciparum* was identified by PCR in 56 (58%). All 56 samples had at least one deletion in pfhrp2, pfhrp3, or flanking regions. Of these samples, 25 (45%) had a deletion in pfhrp2, 39 (70%) a deletion in pfhrp3, and 19 (34%) deletions in both genes. Geometric mean parasite density for the 56 samples was 291/µl and mean complexity of infection was 1.8. Of all positive blood smears in survey subjects, 3.8% appear to have had infections with pfhrp deletions and 1.3% deletions in both pfhrp2 and pfhrp3. Our results suggest that deletions in pfhrp2 or pfhrp3 may explain some false negative malaria RDT results in Uganda, generally in the setting of low parasite densities.

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THE DILEMMAS OF CONGENITAL CHAGAS DISEASE SCREENING IN AN ENDEMIC SETTING

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Chagas disease has shifted from a neglected, endemic parasitic infection of the rural poor to an urbanized chronic disease and now a potentially emergent global health problem. Congenital transmission is estimated to account for 22% of all new *Trypanosoma cruzi* infections. Treatment during infancy is significantly more efficacious and better tolerated, but current diagnostic methods, fail to detect over half of infected neonates and <20% of infants complete 9-months of follow-up. We recruited pregnant women presenting for delivery in two urban hospitals in Santa Cruz department, Bolivia and monitored infants of infected women at birth, 1, 6 and 9 months to evaluate the performance of quantitative PCR (qPCR), IgM Western blots (using TESA-blots) and micromethod (microscopically detectable trypomastigotes) for newborn screening for congenital Chagas disease. Of 518 at-risk infants from 507 seropositive women, unequivocal congenital transmission was identified in 32 infants of 29 mothers, including 3 sets of infected twins (5.7% transmission rate). Vertical transmission was more likely to occur in younger (23.5 years; [CI: 19.6-28.1] vs. [26.9; CI: 22.0-34.0]), first time mothers. Congenital *T. cruzi* infection was significantly associated with severe clinical outcomes including, premature birth (6 vs. 11 infants) and low birth weight (<2500g; 7 vs. 245). Furthermore, uninfected infants of seropositive mothers suffered from respiratory distress (10 vs. 119 infants) and premature labour. In combined birth and 1 month specimens qPCR, TESA-blot and micromethod displayed sensitivity/specificity of 82.8%/97.3% (median of 7143.6 parasites/ml; interquartile range of 5.0-187788.9 parasites/ml), 71.4%/99.5% and 20.7%/100%, respectively. qPCR has the potential to facilitate earlier diagnosis and circumvent loss to follow-up. We critically discuss the technical, logistical and economic obstacles of implementing routine molecular screening for congenital Chagas disease in resource-limited settings and describe the future prospects for improvement.

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TARGETING *TRYPANOSOMA CRUZI* METHIONYL-TRNA SYNTHETASE FOR NOVEL TREATMENT OF CHAGAS DISEASE

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Chagas disease is endemic throughout Latin America infecting approximately 5.7 million people and causing ~7000 deaths per year. New drugs with improved safety and efficacy are needed to replace the antiquated drugs that are currently available. Our group works on a novel drug target in *Trypanosoma cruzi* that is vital for protein synthesis, the methionyl-tRNA synthetase (MetRS). Guided by structure-based drug design, more than 500 novel MetRS inhibitors have been synthesized and tested for activity against recombinant trypanosomal MetRS. The most potent compounds were tested against intracellular *T. cruzi* amastigotes revealing EC50 values as low as 4 nM. The same compounds have minimal toxicity on mammalian cells (selectivity index >5000). Chemical modifications have led to much improved oral bioavailability (up to 80%) and pharmacokinetic properties compared to the parent compounds from which they were derived. A lead MetRS inhibitor was orally administered to mice at 50 mg/kg twice per day for 20 days resulting in no apparent side effects. Experiments testing the efficacy of MetRS inhibitors in the murine model of chronic *T. cruzi* infection are underway. These data support ongoing efforts to develop MetRS inhibitors as novel drugs for Chagas disease.

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NOVEL EXTRACTION PROTOCOL AND RECOMBINASE POLYMERASE AMPLIFICATION ASSAY FOR DETECTION OF *LEISHMANIA DONOVANI* IN 30 MINUTES

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Leishmania donovani (LD) is a protozoan parasite transmitted to humans by sand flies, which causes Visceral *Leishmania*sis (VL). Currently, diagnosis is based on presence of anti-LD antibodies and clinical symptoms. Molecular diagnosis would require real-time PCR, which is not easy to implement at field settings. In this study, we report on the development and testing of a novel extraction protocol in combination with recombinase polymerase amplification (RPA) assay for the detection of LD. The LD RPA assay detected equivalent to one LD genomic DNA. The RPA assay was performed at constant temperature (42°C) and the total assay runtime including the extraction procedure was 30 minutes. The

RPA assay also detected other *Leishmania* species (*L. major, L. aethiopica* and *L. infantum*), but did not identify nucleic acid of other pathogens. Forty-eight samples from VL, asymptomatic and post-kala-azar dermal leishmaniasis subjects were detected positive and 48 LD negative samples were negative by both LD RPA and real-time PCR assays, which indicates 100% agreement. To allow the use of the assay at field settings, a mobile suitcase laboratory (56+45.5+26.5 cm) was developed and operated at the local hospital in Mymensingh, Bangladesh by using a solar-powered battery. DNA extraction was performed by a novel magnetic bead based method, in which a simple fast lysis protocol was applied. Moreover, All reagents were cold-chain independent. The mobile suitcase laboratory using RPA is ideal for rapid sensitive and specific detection of LD especially at low resource settings and could contribute to VL control and elimination programmes.

1265

SPECTRUM OF BACTERIAL PATHOGENS IN INFLAMMATORY CUTANEOUS ULCERS OF AMERICAN TEGUMENTARY *LEISHMANIA*SIS

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Approximately 10% of New World cutaneous leishmaniasis (CL) ulcers manifest with a severe inflammatory phenotype characterized by pain, erythema, and purulent exudate, leading to near-universal treatment with antibiotics prior to anti-leishmanial therapy. Although these ulcers have a "secondarily infected" appearance, the contribution of potential bacterial co-pathogens in the pathogenesis and natural history of severe inflammatory CL is unknown. Understanding the ulcer microbiome in CL has important implications for antimicrobial stewardship, and evidence-based management strategies. Our objective was to illuminate the represented bacterial species in ulcers of CL manifesting with the severe inflammatory phenotype. Pre- and post-antibiotic treatment filter paper lesion impressions (FPLIs) (n=16) from patients with severe inflammatory CL and baseline FPLIs (n=9) from patients with non-inflammatory CL were evaluated using 16S rDNA end-point PCR, 16S real-time PCR and species-specific real time PCR assays targeting *Streptococcus pyogenes, Escherichia/Shigella* spp, *Citrobacter freundii, Enterobacter/Klebsiella* spp, and *Enterococcus* spp. Confirmation to genus and species-level was performed using Sanger sequencing. Six of 16 (37.5%) FPLIs from inflammatory lesions were positive for bacterial pathogens by real time PCR, versus 2 of 9 (22%) FPLIs from non-inflammatory lesions. Six organisms were confirmed to be Staphylococcus spp (n=2), *S. pyogenes* (n=1), *K. pneumonia* (n=1), *Pseudomonas aeruginosa* (n=1), and *Enterobacter* spp (n=1). Two FPLIs retrieved from patients with inflammatory CL post-antibiotic treatment demonstrated pathogen durability. Our findings suggest that, in addition to usual skin flora, Gram-negative enterobacteriaceae and beta-hemolytic streptococci may contribute to the microbiome of severe inflammatory CL ulcers. It remains unknown if empiric antibiotic treatment of the severe inflammatory CL phenotype changes outcome, however, such regimens should include both Gram-positive and Gram-negative coverage.

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CHAGAS DISEASE: A PROSPECTIVE THERAPEUTIC COHORT WITH 12 MONTHS FOLLOW-UP, ANALYZING ADVERSE DRUG REACTIONS, THERAPEUTIC FAILURE AND SEEKING FOR BIOMARKERS"

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Treatment of chronic phase of Chagas disease with benznidazole (Bz) remains controversial mainly because of its weak evidence allowing its use. Its response is variable and presents, commonly, adverse drug reactions (ADRs). There are few studies, describing the ADRs during the treatment of adult patients with Bz and its failure using molecular methods. We established a prospective cohort study that followed, for 12 months, 87 *T. cruzi* infected adults, (age between 18 – 65 years), with the chronic indeterminate form, mild to moderate cardiac or digestive involvement without advanced forms, during Bz use (5mg/kg/day, up to 300mg/day for 60 days). Patients were evaluated in 5 schedules visits for adhesion, ADRs, blood sample collection (near 15th, 30th, 60th days, 6th month and 1st year after Bz initiation). Blood samples were collected to perform biomarkers identification of failure or cure, to perform transcriptomics and mass spectrometry studies. Of the 87 patients that complete the follow-up (FU), 58 (66.7 %) informed at least one ADR, 63.8% dermatological, 41.4% gastrointestinal and 36.2% neurological related. The majority of the ADRs, 45 (77.6%), occurred in the first 15 days of treatment, mainly dermatological related (55.6%). On the 30th visit, 23 (39.7%) patients related ADRs of which, 60.9% were also dermatological related. On the 60th day visit, 27 (46.6%) patients related ADRs, of which, 48.1% neurological related. Regarding the therapeutic efficacy by the PCR (protein chain reaction) positivity for *Trypanosoma cruzi* was assessed (treatment failure was defined as at least 2 positive PCR out 8 replicates). On 60th day of treatment, 15 (17,9%) patients presented positive (+) PCR; up to 6th month, 37 (44.%) presented + PCR and, on the end of the first year after initiation of therapy, from the PCRs analyzed (70% of the total until this date), 40 (66.7%) patients presented +PCR. Although high failure rate demonstrated by PCR, the gold-standard method for cure still being the serology, that may persists reagent for decades, even after effective treatment. Last FU visit was performed on beginning March, 2016 and data is still on final analysis.

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TOWARDS SENSITIVE AND LESS INVASIVE DIAGNOSIS OF VISCERAL LEISHMANIASIS IN SUDAN USING LAMP

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Confirmatory diagnosis of visceral leishmaniasis (VL) usually requires examination by microscopy of samples collected by invasive means, such as splenic, bone marrow or lymph node aspirates, which cause discomfort to patients, with risks of bleeding and iatrogenic infections, and requires technical expertise. Molecular tests such as PCR have great potential for diagnosis of VL using peripheral blood, but are expensive, require well-equipped facilities and trained personnel. More user-friendly, cost-effective, and field-amenable options are therefore needed. One method that could meet these requirements is loop-mediated isothermal amplification (LAMP): amplification of the target occurs at a constant temperature, the reagents are dried down, can be stored at room temperature, is highly specific, and results can be visualized using simple detection methods. A LAMP assay based on dried reagents, developed by Eiken Chemical Co. (Japan), FIND and partners, was evaluated in the diagnosis of VL at the Institute of Endemic Diseases (IEND), Sudan. A total of 198 VL suspects were tested by microscopy of lymph node aspirates (the reference test) and two serological tests: DAT (produced in house at IEND) and *Leishmania* Ab Rapid Test CE (CTK Biotech, USA). LAMP was performed on peripheral blood previously processed by i) a simple direct boil and spin method, and ii) the QIAamp DNA Mini Kit (QIAgen). The sensitivity and specificity obtained for each of the tests was: *Leishmania* Ab Rapid Test CE 98.96% and 100%; DAT 85.57% and 78.22%; LAMP-boil and spin 97.65% and 99.01%; LAMP-QIAgen 100% and 99.01%. The excellent performance of LAMP on blood indicates that it can be included in the algorithm for diagnosis of VL and eliminate the need for invasive lymph node aspirates. The simplicity of the test makes it a promising candidate for confirmatory diagnosis in settings that are lower than the reference laboratory.

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ASSESSING *IN VITRO* AND *IN VIVO* ACTIVITY OF MILTEFOSINE AGAINST *TRYPANOSOMA CRUZI*

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Chagas disease treatment options are limited to Benznidazole (BZ) and Nifurtimox (NFX). These drugs have high efficacy in acute phase but its use in chronic phases is still under debate, and severe adverse effects are frequent. Alternative treatments are not currently available, in part due to high cost of developing new effective molecules. Drug repurposing is a cost-effective potential solution to fulfill current needs of better and safer therapy for Chagas disease. In primary screening at 10 µM, we found that Miltefosine (MLT; a phosphatidylcholine analog with antineoplastic and anti-*Leishmania* activity) exhibited higher relative anti-T cruzi activity (%RA) against trypomastigotes of *Trypanosoma cruzi* VD strain compared to BZ and NFX, and equal %RA against amastigotes. Consequently, MLT was moved to secondary screening, obtaining a dose-response curve with lytic concentration 50% (LC50) of 14.25 µM (CI95%: 8.23; 24.67) on trypomastigotes and inhibitory concentration 50% (IC50) of 1.44 (CI95%: 0.634; 1.716) µM on amastigotes. These results supported further evaluation in an acute murine model of *T. cruzi* infection. Experimental protocol was approved by Faculty of Veterinary Sciences (UBA) (IACUC#2014/4). BALB/c females were infected with 500 trypomastigotes by intraperitoneal (ip) route and treated orally after 8 days of infection with MLT at 25, 50, 75 or 100 mg/kg for 20 consecutive days. Infected non-treated (NT) and BZ or NFX treated groups (100 mg/kg) were included. MLT treatment decreased parasitemia levels in a dose-response manner, with 100% of survival in mice from 50 to 100 mg/kg dosing. Mice with negative parasitemia were subjected to an immunosuppression cycle (cyclophosphamide 200 mg/kg, ip, 1/week, up to four weeks). Parasitemia reactivation was recorded in 100% of mice in all MLT treated groups, as well as in all mice from BZ group, and in 57% of animals from NFX group. MLT exhibited an excellent *in vitro* parasiticidal effect, especially against amastigotes, but parasitostatic activity *in vivo*. Further studies on combined therapies with BZ or NFX and the evaluation of other repurposed candidates are needed and currently performed.

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OPTIMIZING SEASONAL MALARIA CHEMOPREVENTION (SMC) IN AFRICA: ESTIMATING THE IMPACT OF INCREASING THE NUMBER OF SMC CYCLES ON THE NUMBER OF CHILDREN PROTECTED, THE MALARIA BURDEN AND COST-EFFECTIVENESS

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Seasonal malaria chemoprevention (SMC) is currently recommended by the WHO for malaria control in children in areas of the Sahel and sub-Sahel with highly seasonal malaria transmission. SMC consists of up to 4 monthly cycles of sulfadoxine-pyrimethamine plus amodiaquine (SP-AQ) given to all children between 3 and 59 months of age, and offers highly effective protection against malaria. However, large populations reside in areas with seasonal malaria transmission outside the area where SMC is currently recommended, mainly where the rainy season is slightly longer than in the Sahel. Many of these areas have a high malaria burden, despite scale-up of existing control measures. SMC over a longer period could be an important additional control strategy, but evidence on its likely effectiveness, and delineation of the areas where it would be cost-effective are lacking. We used an individual-based malaria transmission model, fitted to data from SMC trials, to estimate and map the impact of SMC with additional monthly cycles across the African continent. It has previously been estimated that around 25 million children live in areas suitable for SMC in areas with a season up to 4 months in length. Model estimates suggest that a wider area, with a population of about 40 million children, could be protected by four monthly cycles of SMC. The population protected by SMC could be further increased, by about 20 million children, by using 5 or 6 rounds of SMC in areas with a longer season, where approximately 60% of the annual burden occurs within a 5 or 6 month period. This could avert approximately 10.5 million malaria cases in young children per year, and many young deaths. These gains are likely to be highly cost-effective, at less than 10 USD per case averted in many areas, due to the relatively low cost of SMC, its high effectiveness where SP and AQ remain effective, and the high malaria burden. Additional monthly cycles of SMC may also be important within the area where SMC is currently recommended, by allowing national programmes to successfully hit the 'moving target' of seasonal cycles which vary in length and intensity from year to year.

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EVALUATION OF THE IMPACT OF SEASONAL MALARIA CHEMOPREVENTION ON MORTALITY AND MORTALITY IN YOUNG CHILDREN IN NORTHERN GHANA

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Malaria remains a major health concern in sub-Saharan African though global burden has reduced from 262 to 214 million from 2000 to 2015. Across the Sahel sub-region, most childhood morbidity and mortality from malaria occurs during the short rainy season. Seasonal Malaria Chemoprevention(SMC) during this period has been shown to prevent illness and death from malaria in children. WHO recommended SMC implementation in the Upper West region of Ghana due to this seasonal pattern. This evaluation was to determine the impact of this implementation. Fourteen communities (clusters) were selected from Lawra District of Upper West Region with 731 children selected as an intervention area with similar selection in the West Mamprusi District(WMD) in the Northern Region with 711 children without SMC for comparison. Blood samples for hemoglobin and malaria parasitaemia were taken before and after the intervention and parameters of malaria morbidity and mortality were also collected during the intervention. Incidence rates of severe malaria were 0.01 and 0.02 per person years follow up in the Lawra District and WMD respectively with P.E of 45%(p=0.62). For mild malaria, it was 0.22 and 0.17 per person years in intervention and control area respectively with PE of - 25%(p=0.31). For children developing anaemia(Hb< 11.0g/dl) from baseline to endline, there was a reduction of 16%(p=0.000) in Lawra and increase of 12%(p=0.002) in WMD. Mean Haemoglobin reduced by 0. 24g/dl(p=0.000) in WMD and increased by 0.39g/dl(p=000) in Lawra District at the end of SMC. At the end of the intervention, proportion of children with asexual parasites reduced by 19%(p=0.000) in Lawra District and increased by12%(p=0.000) in WMD. Morbidity data collection was a challenge in WMD due to health care seeking behaviour and reduced access to health facilities there due to geographical barriers. In summary, effectiveness of SMC in reducing parasite carriage among children in the intervention area has been demonstrated and its protective effectiveness on the haemoglobin level of children.

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EVALUATING THE COMMUNITY-LEVEL IMPACT OF INTERMITTENT PREVENTIVE TREATMENT OF SCHOOLCHILDREN FOR MALARIA IN JINJA, UGANDA: A CLUSTER-RANDOMIZED TRIAL

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Intermittent preventive treatment (IPT) for malaria in schoolchildren has been shown to benefit individual children, and has the potential to decrease malaria transmission at the community level. To evaluate the community-level impact of IPT of schoolchildren with dihydroartemisinin-piperaquine (DP), a cluster-randomized trial was conducted in Jinja, Uganda. A total of 84 clusters, including one primary school and the 100 closest households, were randomized in a 1:1 ratio to intervention and control. Children were enrolled into the intervention (March-Dec 2014) and monthly IPT with DP was delivered (June-Dec 2014), with participants receiving up to 6 rounds of DP. The evaluation included cross-sectional surveys of schoolchildren (Nov-Dec 2014, N=1092) and community members (Jan-April 2015, N=8922), and continuous entomology surveillance in households from 40 randomly selected clusters (April 2014 – April 2015, N=200). In total, 25,630 students were listed on the 42 intervention school registers; 10,079 (39%) were enrolled in the intervention and received at least one dose of DP. Parasite prevalence by microscopy was lower in the intervention arm than in the control in both the school survey (9.2% vs 44.1%, adjusted risk ratio [aRR] 0.22 [95% CI: 0.16-0.30] p<0.001), and the community survey (19.0% vs 23.1%, aRR 0.85 [95% CI 0.73–1.00] p=0.05). Overall, the annual EIR was lower in the intervention arm than in the control (10.9 vs 18.8 infective bites per person/year) and was significantly lower during October-December 2014, when delivery of DP peaked (7.0 vs 24.7, adjusted incidence RR 0.03 [95% CI 0.001-0.46] p=0.01). Despite not reaching coverage targets, we found that IPT with DP had a positive impact on key malaria indicators measured in individual schoolchildren, and within the community. By targeting a demographic 'hot-pop', IPT of schoolchildren provides an operationally attractive option for malaria control that can benefit school-aged children, and potentially reduce transmission of malaria.

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BASELINE FREQUENCIES OF MOLECULAR MARKERS OF DRUG RESISTANCE BEFORE SCALING-UP ACCESS TO SEASONAL MALARIA CHEMOPREVENTION IN SEVEN COUNTRIES ACROSS THE SAHEL

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A major concern for SMC is that its widespread deployment will lead to the selection of drug resistant parasites with progressive loss of efficacy. It is essential that national SMC programmes incorporate a drug sensitivity monitoring component, using standardised methods so that trends over time can be interpreted and data across countries can be combined. Through the ACCESS-SMC project, we aimed to establish a monitoring system that will continue to be used in the longer term to monitor SMC programmes, through monitoring of molecular markers of resistance, and case-control studies. Surveys were conducted in January and February of 2016, in Burkina Faso, Chad, Gambia, Guinea, Mali, Niger and Nigeria, to establish a baseline for the prevalence of molecular markers associated with resistance to sulfadoxine-pyrimethamine and amodiaquine, using standardised methods. Each survey included two age groups, children under 5 years of age, and a group of older children and adults, aged 10 to 30 years, a group not exposed to SMC drugs, for assessment of the extent of changes in the circulating parasite population. The sample size, 2200 in each age group in each country, was chosen to be able to estimate frequencies of the molecular makers with precision and in order to detect changes in frequency that might give early warning of loss of effectiveness when the surveys are repeated in January 2018 and at future intervals. At monitoring locations in each country, where possible in areas that had not started SMC, probability sampling was used to select participants, a short questionnaire was completed to record date of birth, recent antimalarial treatment and other details, and a finger-prick blood sample taken to

make at least two blood spots onto filter paper. These baseline samples are being genotyped to identify mutations at pfcrt, pfmdr1, pfdhfr and pfdhps known to impact on the efficacy of AQ or SP. The baseline survey results will be presented including PCR-determined prevalence of *P. falciparum* and the resistance genotyping data from each country.

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DIHYDROARTEMISIN-PIPERAQUINE FOR SEASONAL MALARIA CHEMOPREVENTION

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Seasonal Malaria Chemoprevention (SMC) using sulfadoxine-pyrimethamine+amodiaquine (SPAQ) is used in the Sahel to prevent malaria in children. Dihydroartemisinin piperaquine (DHAPQ) is an alternative drug that could potentially be used if SPAQ starts to lose efficacy, and in parts of Africa outide the Sahel with seasonal transmission where there are high levels of resistance to SP. We assessed the effect of DHAPQ dose on efficacy as part of a randomized trial to evaluate the use of DHAPQ for SMC in Burkina Faso. 757 children received DHAPQ on three occasions, in August, September and October. Children were weighed each month to determine dosage, which was rounded to the nearest quarter tablet. PQ plasma concentration was measured in capillary samples in a subset of 159 children on day 7 after treatment with DHAPQ. Mean concentration was 48 ng/ml in August, 52 ng/ml in September, and 60 ng/ml in October. To assess the association between PQ concentration on day 7 and protection against malaria during the same month, these children were divided into three groups according to the tertiles of the day 7 concentration, and the incidence compared using a logrank test for trend. Malaria incidence decreased with increasing concentration. The mean total monthly dose of PQ administered was 50 mg/kg. In linear regression, a 10-mg/kg increase in dose of PQ administered was associated with an increase of 4.7 ng/ml in the day 7 plasma concentration of PQ in August, 5.7 ng/ml in September, and 7.7 ng/ml in October, showing an accumulation of PQ in successive months. Cox regression was then used to assess the relationship between the dose of PQ administered and the incidence of malaria in the subsequent month, in the 757 children who received DHAPQ. An increase in PQ dose administered was associated with a reduction in the incidence of malaria, with a hazard ratio of 0.62 for a 10-mg/kg increase in dose administered in August, 0.52 in September, and 0.85 in October. DHAPQ is effective, with the advantage of a fixed dose combination, but it will be important to ensure children receive an adequate dose. There are limited alternatives for SMC if SPAQ fails.

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COMPARATIVE IMPACTS OF ANTENATAL MALARIA PREVENTION STRATEGIES ON *PLASMODIUM FALCIPARUM* SP-RESISTANCE ALLELES IN MALAWI

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The efficacy of intermittent preventive therapy during pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) is continually threatened by parasite resistance to SP. Alternative strategies like intermittent screening and treatment with artemisinin-combination therapies (ISTp) are being considered. We hypothesized that, compared to ISTp with dihydroartemisinin-piperaquine, IPTp-SP would select for higher levels of SP resistance mutations in women infected with *Plasmodium falciparum* at delivery. We analyzed parasites collected from women participating in a randomized trial of ISTp and IPTp-SP at three sites in Southern Malawi, where parasite resistance to SP is high. We pooled *P. falciparum* parasites into populations and sequenced the phenotypically-relevant loci in the parasite genes dhfr and dhps using an Ion Torrent PGM platform. Reads were quality-filtered, aligned to reference sequences, and scored bioinformatically at the loci of interest to compute the frequencies of mutant alleles. Overall, we input 1,410 *P. falciparum* parasitemias into the analysis, comprising 18 pools of between 19 and 104 parasites. After stringent quality-filtering, median read depth was 1,682 at relevant loci in dhfr and 1,552 in dhps. In each population, the frequencies exceeded 97% of the N51I, C59R, and S108N mutations in dhfr and the A437G and K540E mutations in dhps. The I164L mutation in dhfr was absent. The frequency of the dhps A581G mutation was 2.7% in parasites collected at second trimester enrollment. At delivery, the frequency of the A581G mutation in placental parasites was <1% in women who received ISTp and 6.3% in women who received IPTp with SP ($p < 0.001$). This effect was most pronounced at a single study site, where the frequency of A581G in women receiving IPTp-SP increased from 4.3% at baseline to 23.1% in placental parasites ($p < 0.001$). These data indicate that, compared to an ISTp strategy, IPTp-SP promotes the emergence of parasites bearing dhps A581G. Understanding the clinical impact of parasites bearing this mutation on birth outcomes will be critical in areas of East Africa where these parasites circulate.

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EVALUATION OF TARGETED MASS TREATMENT OF MALARIA IN TANINTHARYI REGION, MYANMAR: PRELIMINARY RESULTS

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Targeted mass treatment (TMT) may be a useful intervention for malaria elimination, and is being evaluated for eliminating asymptomatic *Plasmodium falciparum* and *P. vivax* infections detected by active surveillance in malaria endemic populations in eastern Myanmar. A community-based study was conducted in Tanintharyi Region, southern Myanmar to evaluate the feasibility and efficacy of TMT. In preparation, malaria posts were established with trained malaria heath workers, and malaria prevalence was estimated using rapid diagnostic tests (RDT) and ultrasensitive real-time PCR (usPCR) in a total of 35 villages in three rural townships of the Region. Based on the prevalence of subclinical malaria by usPCR, three villages with the highest malaria prevalence were selected to receive TMT, and six control villages were selected. The TMT villages were treated with three daily therapeutic doses of dihydroartemisinin-piperaquine (DHP) and low-dose primaquine, monthly for three months. No treatment was provided in the control villages. Standard diagnosis and treatment for malaria were available in all nine villages. No serious or unexpected adverse effects reported. In pre-TMT screening, only two of 1,750 blood samples collected were positive for *P. falciparum* by RDT, and none were positive for *P. vivax*. By usPCR, the pre-TMT prevalence of malaria ranged from 0-20.8% (*P. falciparum* 0-10.2% and *P. vivax* prevalence 0-18%). The percent reduction for *P. falciparum* was higher in the TMT than the control villages (92.3% versus 72.7%), however the difference was not statistically significant (p-value, xxxxx). The percent reduction in *P. vivax* is significantly higher in the TMT than the control villages (66.7% versus 10.3%, p-value 0.xx). Data for the 12-month follow-up are pending. Results from regression analysis adjusted for a

various covariates will be presented. We conclude that TMT with DHP and low-dose primaquine to eradicate subclinical malaria was well tolerated. The impact of TMT may be greater for vivax malaria than falciparum, but larger studies are needed to differentiate the impact of TMT from seasonal and other variations in prevalence.

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AEDES ALBOPICTUS AT ALTITUDE: WHAT COST FOR CHIKUNGUNYA AND DENGUE TRANSMISSION?

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The spectacular colonization of Europe by *Aedes albopictus* is testimony to its high physiological and ecological plasticity; the species is now present in 27 countries from Spain to Romania and is moving rapidly northward. Although its presence is frequently attributed to importations of used tires in Italy-first detection in 1991-it was already present in Albania in the 1970s. An outbreak of nearly 300 cases of chikungunya in Italy in 2007 and subsequent sporadic autochthonous cases of dengue and chikungunya in other countries confirm the public health significance of the invading species. The strains of the mosquito established in Europe have a marked winter diapause and cold-hardiness, strong evidence that they originated in temperate Asia. If temperature is the limiting factor in its geographic range it could become established as far north as Scandinavia. The critical question is how far north transmission could occur. In this context, the Albanian infestation is of interest because the species is present in isolated mountain villages to at least 1209 m; this allows us to use altitude as a proxy for latitude. We collected eggs at 149, 542, 762 and 1209 m during the summer of 2014 and reared them to F_4 generation. Mosquitoes were orally infected with DENV (serotype 2) and CHIKV (East-Central-South African genotype) at 10^7 FFU/mL and maintained at 28°C, 20°C as well as on a daily cycle of Tmin=15°C and Tmax=25°C. Dissemination and transmission rates were assessed on days 3, 7, 10, 14 and 21 post-infection. We found that titers of CHIKV in saliva compatible with high transmission rates were attained on days 7 and day 10 at 28°C and 20°C respectively. Interestingly, transmission rates for low-altitude strains were higher at 28°C than at 20°C whereas populations collected at high altitude had higher transmission rates at 20°C. By contrast, although all populations became infective with DENV at 28°C, no infection occurred at 20°C. Mean temperatures of 20°C and above are normal for several months in much of Europe. We conclude that if temperature is the key environmental factor limiting transmission, then CHIKV, but not DENV is feasible in much of Europe.

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ASSESSING THE POTENTIAL RISK FACTORS ASSOCIATED WITH NODDING SYNDROME IN NORTHERN UGANDA

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Nodding Syndrome (NS) is a neurological disease of unknown etiology primarily affecting children between the ages of 5 and 15 in a few districts in northern Uganda. NS has been classified as a form of atonic epilepsy and symptoms include uncontrolled head nodding, stunted growth and intellectual disability. Not only does NS cause debilitating symptoms in those affected, but also provokes stigma and unrest throughout affected communities. Although the etiology is unknown, some studies show an association between NS and onchocerciasis, and propose that the vector carrying the causative agent of NS is the *Simulium* spp. black flies. This project aimed to support the hypothesis of *Simulium* spp. as the vector for NS by exploring the prevalence of these black flies in areas affected by NS, and through the creation of a spatial map of potential risk factors. This goal was achieved through two specific objectives. The two objectives of this project were: 1) to better understand and spatially map possible Nodding Syndrome risk factors through household assessments and surveys, and 2) to determine density and distribution through collection and identification of *Simulium* spp. black flies as well as screening of a subset for potential NS pathogens. Demographic and characteristic data was collected from area households both with and without the known presence of a NS case. Black flies were collected, classified, and screened for pathogens. The *Simulium* spp. densities, statistically significant data obtained from the households, and remotely sensed data was mapped atop NS case prevalence data in order to visualize possible patterns and associations of risk factors of the disease. It is our hope that this research will contribute to alleviating suffering in the communities affected by Nodding Syndrome over time by increasing knowledge on this disease and its hypothesized vector.

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ACHIEVING THE VISCERAL *LEISHMANIA*SIS ELIMINATION TARGET IN INDIA WITH EFFECTIVE VECTOR MONITORING

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Visceral leishmaniasis (VL) is a vector-borne neglected tropical disease of public health importance in Bihar and is transmitted by the bite of an infected *Phlebotomus argentipes* sand fly. Since the inception of the VL elimination programme in 1934, indoor residual spraying (IRS) has been conducted with dichlorodiphenyltrichloroethane (DDT) 50% wettable powder (WP). In 2015, in response to evidence showing a rapid decline in *Ph. argentipes* susceptibility, the elimination programme switched to pyrethroid class insecticide, alpha-cypermethrin 5% WP. Entomological monitoring is crucial to ensure insecticide efficacy during the transmission season. Surveys conducted to determine susceptibility status of F1 and field caught *Ph. argentipes* from Bihar showed 100% corrected mortality rates when exposed to 0.1% alpha-cypermethrin and 0.05% deltamethrin. Reduced corrected mortality rates were observed when field caught sand flies were exposed to 0.05% alpha-cypermethrin (87.5-100%) and 4% DDT (24.6-37.5%). To assess the intensity of DDT resistance, CDC bottle bioassays were conducted using F1 *Ph. argentipes*. After 45 minutes of exposure at the diagnostic dose (100µg/bottle) 30% of sand flies were killed and 69% at 10x the diagnostic dose. Mortality breaching the 98% WHO limit for susceptibility was only found at 10x the diagnostic dose after 75 minutes and 5x the diagnostic dose after 150 minutes, demonstrating the intensity of resistance. The efficacy of IRS was assessed by a two stage testing process treating tiles and then artificial walls made of three of the most common surfaces in Bihar (mud, brick and limewash), with either DDT or alpha-cypermethrin. Efficacy of DDT on F1 *Ph.argentipes* sand flies exposed for 30 minutes, was 13.45-21.97% on tiles treated at 1g/m^2, whilst walls treated with the same dose showed mortality of 35.01-67.50%. Observed mortality on alpha-cypermethrin treated walls was within the acceptable WHO range (81.61-90.91%). Monitoring entomological indicators across Bihar provides early indications of sub-optimal impact and could support the programme in achieving its 2017 elimination target.

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SANDFLY OCCURRENCE, DISTRIBUTION AND DIVERSITY IN *LEISHMANIA* ENDEMIC REGIONS IN KENYA

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Phlebotomine sandflies are the only proven vectors of visceral leishmaniasis (VL) but knowledge of their distribution and diversity in Kenya is partial. Accurate knowledge of this is fundamental to implementation of vector control strategies. We explored the occurrence, distribution and diversity of sandflies in leishmaniasis endemic areas in Kenya. Vector sampling was done from three Counties: Baringo, Nakuru and Marsabit. Leishmanaises hot spots were identified using health facility screening and treatment records. Trapping was done indoors and outdoors around termite mounds, vertisols, sleeping areas, animal sheds and burrows using CDC light traps and sticky papers. Morphological and molecular identification of vector species was done using taxonomic keys and sequencing of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene. Dissected midguts of sandflies suspected to harbor promastigotes were cultured in NNN media overlaid with complete Schneider's media or blood agar. Vector density from each collection site was recorded. 14,000 sandflies were collected: Baringo (52.3%), Nakuru (8.6%), Marsabit (37.2%). Overall 40% were *Phlebotomus martini*. In Baringo, Marigat sub-county, *P. martini* was the only vector trapped while in East Pokot *P. duboscqi* (40%) and *P. martini* (30%) were the most prevalent. In Gilgil (Nakuru), *P. guggiesbergi* (60%) was prevalent, *P. saevus* (6%) and *P. sergenti* (5%) were also identified. In Marsabit, two main vectors were collected, *P. martini* and *P. orientalis*. Outdoor trapping yielded the highest number with *P. martini* , *P. orientalis* and *P. duboscqi* found both indoor and outdoor. 95% of the *P. orientalis* were from acacia dried swampy cracked soils areas while *P. duboscqi* was collected along the river bed. Presence of *P. duboscqi* in East Pokot, here identified for the first time, could indicate the presence of cutaneous leishmaniasis. *Leishmania*sis transmission could be occurring outdoors as many people slept outside. Vector control strategies should target both indoor and outdoor settings. Vector studies add to understanding dynamics of leishmaniases' transmission which is important for disease control.

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EVOLUTION OF *GLOSSINA FUSCIPES* S.L IN HUMAN AFRICAN TRYPANOSOMIASIS *FOCI* - EVIDENCE FOR CRYPTIC SPECIES

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Human African Trypanosomiasis (HAT) or sleeping sickness is a neglected parasitic disease endemic to rural sub Saharan Africa. Gambiense HAT which accounts for more than 95% of all HAT cases has been targeted for elimination by 2030 through the scale up of novel, and highly cost-effective, vector control tools such as 'tiny targets' in combination with drugs. However there are long standing gaps in our understanding of the heterogeneity of HAT transmission and the ecology of tsetse flies (genus *Glossina*). HAT foci have historically remained spatially stable despite the high mobility of both the host and the vector. We hypothesised that some of this spatial heterogeneity in HAT may be driven in part by vector population structure. Information on the evolutionary relationships within and among populations of *G. fuscipes* s.l that accounts for >90% of HAT transmission is limited. We carried out a preliminary study using both mitochondrial (cytochrome oxidase sub unit 1 (CO1), NADH dehydrogenase sub unit 2 (ND2) and 16S ribosomal (16S)) and nuclear (internal transcribed spacer 1 of ribosomal (ITS1)) DNA markers to examine the phylogenetic relationships within *G. fuscipes* s.l in HAT foci in Uganda and the Democratic Republic of Congo, with a particular emphasis on *G. f. fuscipes* and *G. f. quanzensis*. Our results suggest that there is marked interspecific divergence between these two sub-specific forms. Further, in areas where there is a single sub-species we have generated evidence of marked differentiation between sub-populations and even within sampling locations. The public health importance of these preliminary findings is vector diversity may be contributing to the epidemiological complexity of HAT transmission and its control.

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SPATIO-TEMPORAL ANALYSIS AND *TRYPANOSOMA CRUZI* (AGENT OF CHAGAS DISEASE) INFECTION PREVALENCE OF CITIZEN-COLLECTED TRIATOMINE VECTORS ACROSS THE SOUTHERN USA

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Due to increased reports of local transmission and widespread media and public interest in Chagas disease in the southern United States, defining the spatial and temporal occurrence of triatomine vectors and their infection with *Trypanosoma cruzi* is critical for public and veterinary health protective measures. Through a citizen science program and field collections from 2013 to 2015, we collected 2,812 kissing bugs from diverse ecological regions in Texas, as well as 66 bugs from 7 additional southern states. The majority of citizen-collected bugs were found in homes, kennels, patios, or other peridomestic settings. Using a combination of morphological and molecular identification, we identified 7 different species of *Triatoma*. Most commonly (97% of adults), triatomines were encountered between May and October. The two most common species, *T. gerstaeckeri* and *T. sanguisuga*, exhibited activity peaks in mid-summer and early fall, respectively. A point pattern analysis revealed unique geographic occurrences of the different *Triatoma* spp., suggesting that suitable habitat varies among the triatomine species. Using real-time PCR to detect *T. cruzi* DNA in bug hindguts, we found an overall *T. cruzi* infection prevalence of 63%. *T. cruzi* infection prevalence varied among triatomine species, ranging from 29% (95% CI: 19-39%) in *T. rubida* to 69% (95% CI: 65-73%) in *T. gerstaeckeri*. Parasite lineages revealed through strain typing were TcI (43%) and TcIV (57%). These results demonstrate wide-spread occurrence of triatomine bugs in Texas, with ubiquitous infection with *T. cruzi* infection of strain types TcI and/or TcIV. However, heterogeneity existed in triatomine species' spatial and temporal occurrence, and infection with different lineages of *T. cruzi*. Consideration of local temporal and spatial heterogeneity of *Triatoma* spp. occurring in Texas will allow targeting of vector control and medical/veterinary outreach initiatives to reduce human and animal vector contact.

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BARCODED LIVE ARTHROPOD SCREENS FOR HIGH THROUGHPUT DISCOVERY OF NOVEL VECTOR CONTROL AGENTS

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To accelerate the search for new vector control agents we have developed a high throughput screening method for phenotypic screens on live arthropods. This new method relies on DNA-barcoding to trace individual insects during experiments. To identify novel mosquitocidal agents, we mixed DNA-barcoded microspheres with a bloodmeal and

test compound prior to membrane feeding of *Anopheles stephensi* on 96-well plates. Each well contained a unique barcode and test specimen. Twenty-four hours post feeding we pooled dead mosquitoes and used PCR amplification and Luminex-based multiplex detection of barcode sequences to identify compounds with mosquitocidal activity. Similarly, the fraction of live mosquitoes was analyzed to assess sampling of every well. Plate feeding was very efficient, with over 90% of fed mosquitoes and minimal cross-feeding between different wells. The barcoding approach reliably detected positive control compounds that were spiked in different wells on the plate. Screening of a chemical library identified a number of compounds with potent adulticidal activity against *Anopheles*. Two of these compounds appeared to have excellent pharmacokinetic properties in Beagle dogs and showed plasma levels well above the IC90 for more than eighty days at well-tolerated doses. These compounds are promising candidates for development of mosquitocidal drugs for human or veterinary use. We explored other barcoded screening modalities and have generated proof of concept for repellent and attractant screens, which lead to an underrepresentation and overrepresentation of sample barcodes, respectively. Using this technique we can screen 96 different test specimens on a single container of mosquitoes, which exceeds the current throughput capacity substantially and will significantly contribute to discovery and optimization of novel vector control agents.

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A RANDOMIZED CONTROLLED TRIAL OF THE NEUROPSYCHOLOGICAL BENEFITS OF COMPUTERIZED COGNITIVE REHABILITATION TRAINING IN UGANDAN CHILDREN SURVIVING SEVERE MALARIA

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We have previously documented persisting neurocognitive deficits from cerebral malaria (CM) and severe malaria anemia (SMA). In this RCT, 150 Ugandan CM/SMA survivors (two years after illness) 5 to 12 yrs old and 150 non-malaria children from their households were randomly assigned to 3 treatment arms. One treatment arm was computerized cognitive rehabilitation training (CCRT) with 9 games for enhancing attention, working memory, and visual-spatial processing in which game difficulty increased with proficiency. The limited CCRT arm was the same only with training cycling through simpler levels of difficulty. Children in the passive control arm received no CCRT. Before and after 24 sessions of training over 8 weeks, and one year following training, children were evaluated with tests previously used to establish persisting neurocognitive deficits for CM/SMA. These were the Kaufman Assessment Battery for Children, 2nd ed. (KABC-2), computerized Tests of Variables of Attention (TOVA), computerized CogState cognitive tests, the Behavior Rating Inventory for Executive Function (BRIEF; caregiver rating), and the Achenbach Child Behavior Checklist (CBCL; caregiver rating). Malaria survivors receiving either full or limited CCRT showed significant improvements (compared to passive controls) on KABC-II Mental Processing Index (MPI; composite of all scales), visual-spatial processing (VSP), and the executive functioning (EF) test of conceptual reasoning; persisting to 1-yr follow-up only for VSP. BRIEF and CBCL behavior measures significantly improved, but not until 1-year post CCRT. Non-malaria children receiving CCRT benefited on KABC-II Story Completion (EF), not persisting to 1-yr follow-up. CogState maze chase (visual-motor tracking/attention), and maze learning improved, and these benefits continued at 1-yr follow-up. We present RCT evidence that CCRT is viable for evaluating and enhancing some domains of cognitive performance in brain-injured children in resource-limited settings. However, additional follow-up CCRT intervention components are needed for extending the long-term benefits for clinical populations.

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ASSESSING THE POTENTIAL TOXICITY HAZARD TO AQUATIC LIFE FROM IMMERSION OF INSECTICIDE-TREATED MOSQUITO NETS DURING FISHING AND WASHING

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The combination of efficacy and a favorable environmental and human health profile has made the pyrethroid insecticides a mainstay of malaria vector control. Pyrethroids are extremely toxic to fish and other aquatic life, but appropriate indoor usage is unlikely to contaminate lakes, rivers, or coastal environments. Long-lasting insecticidal nets (LLINs) were expected to mitigate the potential environmental impacts of bednets by eliminating the need for on-site (re)treatment and thus the possibility of concentrated insecticide dipping solutions ending up in local water bodies. However, LLINs have brought new concerns: enhanced durability makes these nets appealing for misuse as fishing gear. Furthermore, LLINs contain more insecticide than conventional treated nets, but local practices for washing nets - often in a nearby lake or stream - have not changed. We present a rigorous, quantitative assessment of the potential toxicity hazard to aquatic life from LLIN immersion during washing or fishing. Fourteen LLINs currently recommended by the WHO were included in our analysis. We aggregated existing data on insecticide release from nets, physicochemical properties of pyrethroids, and environmental breakdown pathways, as well as on pyrethroid toxicity. A model was built to simulate insecticide release and persistence in the environment following immersion of LLINs. Aggregated data were used to bracket concentrations and environmental transformations for a number of simplified, hypothetical scenarios. The results were compared to toxicity data to determine the potential threat to aquatic organisms. Although limitations in the currently available data precluded a full risk assessment, our results suggested that there is evidence to support concerns that immersion of LLINs could pose a threat to aquatic life, particularly in small water bodies or areas with limited circulation. Aquatic macroinvertebrates may be at greater risk than fish species. This is the first assessment to consider the potential ecotoxicological impacts from LLINs in such quantitative terms, including sorption, net-, and compound-specific interactions.

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EVALUATING THE IMPACT OF THE NATIONAL SCALE-UP OF MALARIA CONTROL INTERVENTIONS IN LIBERIA FROM 2004 TO 2013

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Beginning in 2004, after the civil war, significant investments were made to expand malaria control and prevention in Liberia. To assess these efforts, an impact evaluation was conducted using a pre and post design with a plausibility assessment. Trends in all-cause childhood mortality (ACCM) were analyzed against trends in coverage of malaria control interventions and contextual factors that affect child survival. Data from Demographic and Health Surveys, Malaria Indicator Surveys, and the health information system were used in the evaluation. Household coverage of at least one

insecticide-treated bednet (ITN) rose from 30% in 2007 to 55% in 2013. Increases were observed in ITN use among children under-five (26% to 38%), pregnant women (33% to 37%), and the general population (23% to 32%) from 2009 to 2013 (no previous data available). Coverage of intermittent preventive treatment in pregnancy (two or more doses of sulfadoxine-pyrimethamine), introduced in Liberia in 2005, rose to just under 50% by 2013. Care seeking for children with fever remained stable during the evaluation period (65% in 2007 and 69% in 2013), while diagnostic testing for malaria among children with fever rose from 24% in 2009 to 42% in 2013, and treatment with first-line antimalarials increased from 13% in 2007 to 40% in 2011. ACCM gradually declined during this same period from 109 (95% CI: 99-120) in 2007 to 94 (95% CI: 84-103) deaths per 1,000 live births in 2013; however, this decline was mainly due to a decrease in infant mortality from 71 (95% CI: 62-80) to 54 (95% CI: 46-61) deaths per 1,000 live births as child mortality (mortality between age 1 and 4 years) remained stable during this period (41 and 42 deaths per 1,000 live births in 2007 and 2013, respectively). The evaluation period occurred within an overall environment of improvement in the country post-civil war, where the healthcare system was being rebuilt, GDP was rising, and other improvements in maternal and child health were taking place. The gradual expansion of malaria control interventions may have partially contributed to the decrease in mortality, but other factors also likely contributed to the decline.

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THE RELEVANCE OF OUTDOOR RESTING AND SLEEPING FOR BED NET USE IN THE GAMBIA

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Despite the level of coverage of Long-Lasting Insecticide-treated Nets (LLIN) and Indoor Residual Spraying (IRS) has steadily increased, malaria persists in The Gambia. This persistent transmission offers new challenges for malaria elimination. In order to gain further understanding in the human-vector interaction and its effect on malaria control, a social science study assessed human activity, resting and sleeping behaviour during the evening and at night in rural Gambia. Using a sequential mixed-methods study design, quantitative survey data and the direct observation of bed net use and evening activities (n=201) were complemented with qualitative research. Out of 76.1% respondents who slept under a mosquito net, 57.5% were adequately protected from malaria by the nets. In addition to net availability, net use was affected by sleeping patterns inside and outside the house. Adolescents (57.2% male and 50.2% female) and adults (57.2% male and 51.2% female) often socialize, rest or sleep unprotected outside their houses during the early evenings (18.00-21.00). Of the respondents, 16.4% moved between different resting and sleeping spaces during the evening, potentially leading to higher exposure to malaria. Qualitative data showed that specific subgroups are unlikely to use their bed nets, such as farmers protecting crops, herding cattle, burning charcoal or hunting during the evening. These findings suggest that unprotected resting or sleeping and evening activities might contribute to on-going transmission.

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COST-EFFECTIVENESS OF SEASONAL MALARIA CHEMOPREVENTION IN UPPER WEST REGION OF GHANA

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In Ghana, malaria is endemic and perennial (with significant seasonal variations in the three Northern Regions), accounting for 33% of all deaths among children under-five years, with prevalence rates in children under-five years old ranges from 4% in Greater Accra to 51% in Upper West Region. Ghana adopted the WHO-recommended Seasonal Malaria Chemoprevention strategy with a trial in the Upper West Region in 2015. The objective of this study was to estimate the cost-effectiveness of SMC. Costs were analysed from the provider and societal perspectives and are reported in 2015 USD. Data on resource use (direct and indirect costs) of the SMC intervention were collected from intervention records and a survey in all districts and at the regional level. Additional number of malaria cases and deaths averted by the intervention were estimated based on prevalence data obtained from an SMC effectiveness study in the region. Incremental cost-effectiveness ratios (ICERs) were estimated for the districts and region. Sensitivity analyses were conducted to test the robustness of the ICERs. The total financial cost of the intervention was US$1,142,040.80. The total economic cost was estimated to be US$7.96 million and US$2.66 million from the societal and provider perspectives respectively. The additional number of cases averted additional deaths estimated to be averted by the intervention were 24,881 and 808 respectively. The economic cost per child dosed was US$67.35 from societal perspective and US$22.53 from the provider perspective. The economic cost per additional case averted was US$107.06 from the provider perspective and US$319.96 from the societal perspective. The economic cost per additional child death averted by the intervention was US$3,298.36 from the provider perspective and US$9,858.02 from the societal perspective. The financial cost per the SMC intervention delivered to a child under-five was US$9.66. The ICERs were sensitive to mortality rate used. The SMC intervention is economically beneficial in reducing morbidity and mortality in children under-five years and presents a viable approach to improving under-five health in Ghana.

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EXPANDING THE TOOLBOX: A SYSTEMATIC REVIEW LOOKING AT OLD AND NEW VECTOR CONTROL TOOLS

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To achieve the new global targets for malaria elimination, additional vector control tools (VCTs) are critical to supplement long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS). We conducted a systematic review and expert consultations to identify VCTs with existing or future potential to reduce malaria transmission, to assess their readiness for implementation and to identify gaps in the supporting evidence. In consultation with expert groups, a total of 22 malaria VCTs were identified *a priori*, including larval source management, topical and spatial repellents, attractive toxic baits and endectocide treatment of humans and livestock, among other interventions. Six electronic databases and grey literature sources were searched from 1 January 1980 to 28 September 2015 to identify systematic reviews, Phase I-IV studies and models that assessed

the effect of each VCT on epidemiological and/or entomological outcomes across all age groups in all malaria-endemic settings. Eligible studies were summarized qualitatively and quality and risk of bias assessments undertaken where relevant. Recommendations of the Preferred Reporting Items for Systematic Reviews group were followed. Operational readiness and potential for impact were additionally assessed through consultations with field experts and national malaria control program managers. We will present search results and compare the current availability and quality of evidence (for systematic reviews and Phase III studies) for each VCT, in addition to the findings of the expert consultations on the relative potential of existing and nascent malaria VCTs to contribute to malaria elimination. Identifying VCTs to supplement LLINs and IRS will be central to global malaria elimination and eradication efforts.

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COMMUNITY HEALTH WORKERS' PERCEPTIONS OF AND SATISFACTION WITH THEIR ROLE IN IMPLEMENTING A COMMUNITY CASE MANAGEMENT FOR MALARIA PROGRAM: IMPLICATIONS FOR FEASIBILITY AND SCALE-UP

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Community case management for malaria (CCMm) programs target prompt diagnosis and treatment of the disease in populations with inadequate access to formal health facilities. The World Health Organization recently launched the Rapid Access Expansion Programme to scale-up integrated community case management (iCCM), a strategy which addresses other childhood illnesses in addition to malaria. The feasibility and sustainability of many CCMm and iCCM programs depend largely on community health workers (CHWs) consistently providing high-quality services. We implemented a CCMm program which provides training and support (but no direct compensation) for established CHWs to provide free rapid diagnostic tests for malaria (mRDTs), along with results-conditional coupons redeemable for discounted antimalarials at retail shops. Assessing CHWs' perceptions of and satisfaction with their volunteer role is essential to maintaining high-quality performance, identifying opportunities for program improvement, and demonstrating the potential for replication and scale-up. We therefore surveyed participating CHWs to determine the intrinsic and extrinsic factors that motivate (or conversely, discourage) their sustained involvement in providing CCMm services. Following training, 274 CHWs out of 287 had acceptable performance and were provided with commodities to perform mRDTs. Over the first six months of the program, six CHWs discontinued participation or were not invited to continue participation based on performance. We implemented a structured questionnaire to assess CHWs' perceptions of, and satisfaction with, their CCMm role as well as any perceived externalities of participation on their other activities and responsibilities both within and outside the context of their CHW position. The questionnaire captured diverse aspects of CHWs' perceptions of their role with regards to internal motivation, effectiveness, sustainability, community trust, and logistics. Our findings have implications for the sustainability and scale-up potential of CCMm and iCCM, especially for models dependent on a volunteer workforce.

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DISEASE-SPECIFIC CYTOKINE PROFILES IN PEDIATRIC PATIENTS WITH MALARIAL, HIV, AND SYSTEMIC BACTERIAL INFECTIONS

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We present 680 cytokine profiles of blood drawn from well-characterized pediatric malaria patients in the high-disease-burden environment of Siaya, Kenya. High levels of co-infections were observed in this group, including HIV and bacteremia from non-Typhoidal Salmonella (NTS) and *Staphylococcus* sp. Cytokine profiles were placed into one of nine disease categories, including healthy controls, reflecting the bulk of seriously ill suspected malaria patients accepted into our study at the Siaya County Hospital. Distinct cytokine signatures were identified using LASSO, a model selection algorithm. Bootstrapping of the model selection procedure provided robustness to our answers against artifacts arising from the complexity of our data. Linear models using selected cytokines were able to identify comorbidities as the most important complexity impacting the cytokine profile. We were able to distinguish bacteremia from malaria with ROC areas under curve of 0.98, 0.85, and 0.88 for differentiating mono-infection with NTS, co-infection of NTS with malaria, and mono-infection with Staphylococcus bacteremia, from mono-infection with malaria, respectively. Uninfected controls could be distinguished from the malaria background with an AUC of 0.91. IL-7, IL-8, TNFα, and MIG were the most informative cytokines for distinguishing NTS bacteremia from malaria, while IL-10 and IL-7 were the most able to distinguish Staphylococcal bacteremia from malaria. Progression of malaria to SMA was indicated by high levels of IL-2R and low levels of IP-10, while malaria was distinghished from the healthy controls with IL-2R, IL-10, MIP1b, and RANTES. Additional significant correlations of cytokine profiles with death (IL-8), malnutrition (IL-8, IP-10, TNFα, and IL-15), respiratory distress (Eotaxin and IL-8), high fever (IL-6, IP-10, and IL-10), hemoglobin level (IL-2R, IL-6, and RANTES), age (IL-1b, IL-2R, and Eotaxin), and the reticulocyte production index (RANTES and TNFα), were observed.

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COMPARISON OF THE INCIDENCE OF ACUTE LEPTOSPIROSIS IN THE KILIMANJARO REGION OF TANZANIA BETWEEN 2007-08 AND 2012-14

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The sole report of annual leptospirosis incidence in continental Africa of 75-102 cases per 100,000 population is from a study performed in August 2007 through September 2008 in the Kilimanjaro Region of Tanzania. To evaluate the stability of this estimate over time, we estimated the incidence of acute leptospirosis in Kilimanjaro Region, northern Tanzania for the time period 2012-2014. Cases were identified among febrile patients at two sentinel hospitals in the Kilimanjaro Region. Leptospirosis was diagnosed by serum microscopic agglutination testing using a panel

of 20 serovars belonging to 17 separate serogroups. Serum was taken at enrolment and patients were asked to return 4-6 weeks later to provide convalescent serum. Confirmed cases required a 4-fold rise in titer and probable cases required a single titer of ≥800. Findings from a healthcare utilization survey were used to estimate multipliers to adjust for cases not seen at sentinel hospitals. Among 1,115 patients presenting with fever, 19 (1.7%) had confirmed or probable leptospirosis. Of cases, the predominant reactive serogroups were Australis 8 (42.1%), Sejroe 3 (15.8%), Grippotypphosa 2 (10.5%), Icterohaemorrhagiae 2 (10.5%), Pyrogenes 2 (10.5%), Djasiman 1 (5.3%), and Tarassovi 1 (5.3%). We estimated the annual incidence as 11-18 cases per 100,000 population. We estimated a much lower incidence of acute leptospirosis than previously, with a notable absence of cases due to the previously predominant serogroup Mini. Our findings support the value of multi-year surveillance to understand leptospirosis epidemiology.

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FINE-SCALE GPS TRACKING TO QUANTIFY HUMAN MOVEMENT PATTERNS AND EXPOSURE TO LEPTOSPIROSIS IN THE URBAN SLUM ENVIRONMENT

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Understanding fine-scale movement of individuals is critical for understanding the dynamics of environmentally transmitted diseases, such as leptospirosis, for which the mechanisms underlying proxy associations with environmental sources of contamination are unknown. We recruited male and female participants from an ongoing cohort study in an urban slum community in Brazil with high leptospirosis infection rates (6.3% and 3.4% per year, respectively). We conducted 24 hours of GPS tracking at 30 second intervals, which resulted in data with a fine temporal and spatial resolution. We validated the data with diaries and exit interviews. GPS data were cleaned with a velocity filter and analyzed to estimate activity spaces and time spent in proximity to the household and to transmission sources for leptospirosis. Among the 172 recruited cohort subjects, 130 agreed to participate and 100 wore the GPS for a full 24 hours. Both male and female participants spent the majority of their timepoints near their residence (male mean 81.2%, female mean 85.0% within 50 meters of the home, p = 0.32) and within the slum community (male mean 86.4%, female mean 88.3%, p = 0.58). However, males had a significantly larger activity space during the sampling period than did females (61034m2 vs 42101m2, p = 0.015). The activity space within the urban slum environment was characterized by high density of open sewers, flood-prone regions, and rat activity as ascertained by tracking board studies. In summary, we found that slum residents spend most of their time near their households, indicating that exposures to leptospirosis occur in the environment where they reside. The finding that males visited a larger area within the peridomestic environment may explain the high infection rates observed in this group. GPS tracking therefore is able to delineate fine-scale movement patterns within complex slum environments, provide useful insights into the mechanisms of environmental exposures, and identify opportunities for targeted prevention.

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POSTPARTUM INFECTION AT A UGANDAN REGIONAL REFERRAL HOSPITAL: MICROBIOLOGY AND ANTIMICROBIAL RESISTANCE PATTERNS

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The World Health Organization estimates that puerperal sepsis causes 10% of maternal deaths in Africa, but prospective studies on sepsis incidence, microbiology and outcomes are scant. We performed a prospective cohort study of 4,235 Ugandan women presenting to a regional referral hospital for delivery or postpartum care, measured vital signs after delivery, performed microbiologic evaluation of all febrile and hypothermic women, and followed them until 6 weeks postpartum by phone. Mean age was 24.9 years, 487 (12%) were HIV-1 infected, and 50% had cesarean delivery. Temperature was measured for 4179 (99%); 201 (4.8%) were febrile or hypothermic, and blood and urine samples were collected from 186 (93%). Of these, 58 (31%) had infection confirmed, 52 (90%) of whom were febrile. Five (8.6%) had malaria, 5 (8.6%) had bloodstream infection, 7 (12%) had pyelonephritis, 13 (22%) had catheter-associated urinary tract infection, and 43 (74%) had postpartum endometritis. Postpartum infection incidence did not differ by delivery mode or HIV status. Of 5 bloodstream infections, 3 were Gram-negative rods (GNRs, *S. typhi*, *Acinetobacter*, and *E. coli)*, of which 2 demonstrated extended-spectrum β-lactamase (ESBL) phenotype. Of 7 women with pyelonephritis, 5 associated organisms (71%) were GNRs, all of which (100%) were ESBL phenotype. Of 13 catheter-associated urinary tract infections, 8 organisms (62%) were GNRs (6 *Acinetobacter* spp. and 2 *E. coli*), 7 of which (88%) were ESBL phenotype. Maternal mortality incidence was 0.06% in-hospital and rose to 0.26% by 6 weeks postpartum. Combined stillbirth and neonatal mortality incidence was 4.7% in-hospital, rose to 5.9% by 6 weeks postpartum, and did not differ between women with or without postpartum infection (*P*=0.17 in-hospital, *P*=0.32 at 6 weeks). Here, we demonstrate infection is common among febrile Ugandan women hospitalized for delivery or postpartum care. The microbiology of urine and bloodstream infections is dominated by antibiotic-resistant Gram-negative rods. Increasing availability of microbiology testing to inform appropriate antibiotic use should be a high priority in this setting.

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EMERGING PATHOGENIC BACTERIUM *ELIZABETHKINGIA ANOPHELIS*: DIVERSE MOBILE GENETIC ELEMENTS ARE PRESENT ACROSS STRAINS AROUND THE WORLD

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Genus *Elizabethkingia* was separated from *Chyseobacterium* in 2005, with two member species, *E. meningoseptica*, a notorious pathogen causing meningitis, and *E. miricola*, an isolate obtained from condensation water of Space Station Mir. The third species, *E. anophelis* was originally isolated from the gut of *Anopheles* mosquitoes and described in 2011. In 2013, *E. anophelis* caused human infections were documented in Central Africa, and an outbreak in an intensive-care unit in Singapore was reported. In 2015, evidence came to light that *E. anophelis* infections could be transmitted from mother to infant. In 2016, an *E. anophelis* outbreak occurred in Wisconsin. The genome annotation revealed a large genetic capacity of the antibiotic resistance, defense against oxidative stress and TonB dependent transporters. Those features may contribute

to the virulence and pathogenesis. In this study, we reciprocally compared genomes of mosquito isolates and human isolates in different geographic locations including Central Africa, Singapore, Hong Kong, China and Wisconsin, in an attempt to recognize mobile DNA elements that may provide clues to identify genome signature that is related to pathogenesis. The comparison revealed the presence of variable discrete integrative conjugative elements (ICE) across the isolates. These ICEs contain genes that are involved in pathogenicity functions such as antibiotic resistance and virulence. The ICEs in mosquito isolates were degenerated with missing necessary Tra genes for mobility. Interestingly, a Type II CRISPR cas unit was identified in the pathogenic isolate from Central Africa. The genome comparison provided valuable information for further studies of pathogenesis, ecology and evolution of the species.

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FAILURE OF STRAIN-SPECIFIC IMMUNE INDUCTION TO GROUP A STREPTOCOCCUS MAY UNDERLIE THE EPIDEMIC OF STREPTOCOCCAL PYODERMA: OVERCOMING IMMUNE RESISTANCE THROUGH VACCINATION

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The epidemic of streptococcal pyoderma is responsible for Australia's Indigenous populations suffering the highest rates of rheumatic heart disease worldwide. The cause of the epidemic is poorly understood from an immunological perspective. There are in excess of 200 different strains of group A streptococcus (GAS) based on sequence differences in the M protein and it is known that antibodies to the serotypic amino-terminal segment of the protein can kill organisms *in vitro*. It is assumed that M protein sequence diversity is solely responsible for the prolonged period of time required to develop immunity, with immunity developing to common strains one at a time as a result of individual infections. We used four endemic pyoderma strains of GAS from patients in the Northern Territory to model the acquisition of natural immunity. Surprisingly, infection with one strain led to short-term protection only against a challenge infection with that particular strain. Immunological memory did not develop. Two sequential infections with the same strain were required to induce enduring strain-specific immunity. Sequential infections with different strains resulted in partial short-term immunity and only to the last strain to which the mice had been exposed. Mice exposed to multiple strains, either sequentially or simultaneously, did not develop antibodies to a conserved M protein vaccine peptide, J8, demonstrating that this epitope is cryptic to the immune system. However, in contrast to the lack of strain-specific immunity that follows infection, immunity following vaccination with J8 protects against multiple strains delivered sequentially or as a co-infection. Moreover, vaccine-mediated immunity was maintained following sequential heterologous infections. This study describes a major reason why immunity to GAS pyoderma is so slow to develop, but also shows that vaccination with a conserved peptide vaccine can prevent infections with multiple strains.

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HOUSEHOLD MODELLING OF YAWS DATA INDICATES THAT TARGETING TREATMENT USING CASE FINDING AND CONTACT TRACING MAY BE UNSUCCESSFUL AT ERADICATING THE DISEASE

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Yaws is a painful and disabling infectious disease that causes skin and bone lesions. It is most commonly seen in children in warm, humid tropical areas. Individuals with the disease can be effectively treated with a single dose of oral azithromycin. Yaws is known to be clustered, even within endemic regions, but the spatial epidemiology remains poorly understood. Previous eradication campaigns used either mass treatment with benzathine-penicillin or more targeted treatment of cases and their contacts, depending on the prevalence of clinical disease. As many individuals are latently infected without clinical evidence of disease, this often resulted in failure to adequately treat latently infected individuals, resulting in subsequent rebound in disease incidence and ultimately failure of the eradication programme. The new WHO strategy mandates an initial round of MDA followed either by further MDA or active case finding and treatment of cases and their contacts. Multiple rounds of MDA can be expensive and requires large amounts of antibiotics, which are not currently donated for yaws eradication. We employ a household model to study the transmission of the disease using data collected from a pre-Mass Drug Administration (MDA) survey conducted in the Solomon Islands. We used this model to assess whether targeting cases and their household contacts would be sufficient to interrupt transmission. Our data indicate that a limited number of rounds targeting cases and their household contacts would leave 75% of latently infected individuals untreated, leading to the need for either many more rounds of treatment or a broader treatment strategy to successfully break transmission.

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LONG-TERM EFFECT OF MASS DRUG ADMINISTRATION FOR SCABIES IN FIJI: EXPERIENCE FROM THE SHIFT TRIAL

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Control of scabies based on treatment of individual cases is difficult because of frequent re-infestation. We implemented a community intervention trial of mass drug administration (MDA) for scabies to ascertain the efficacy and safety of two alternative regimens (topical permethrin and oral ivermectin MDA), compared with standard care. We identified three isolated island communities in Fiji and randomly assigned one of the three treatment regimens: ivermectin MDA, permethrin MDA or standard care with permethrin. All participants were sought for re-examination at 12 months, and at 24 months via a 20% sample. The study enrolled 2051 people. At baseline, scabies prevalence was high in all arms (32.1%, 41.7% and 36.6% in the three arms respectively). After one year the prevalence of scabies, previously reported, fell to 1.9% in the ivermectin arm corresponding to a reduction in prevalence of 94%. Scabies prevalence was also reduced to a lesser extent in the two other arms. At two years, scabies prevalence in the ivermectin arm was 3.7%, compared to 13.4% and 15.4% in the other two arms. In conclusion, the effect of MDA, particularly with ivermectin, was long lasting, with very low prevalence maintained even after two years following administration.

The study was the first to compare MDA for scabies with the conventional approach of treating symptomatic cases and contacts, and the first to undertake two year follow up. This strategy is likely to be highly beneficial for communities where this disease is endemic.

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PLAGUE IN MADAGASCAR: LIMITING THE TRANSMISSION BY IMPROVING THE CONTROL OF *XENOPSYLLA CHEOPIS*, THE MAIN FLEA VECTOR OF *YERSINIA PESTIS*

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Madagascar is the second country most affected by plague worldwide, with around one thousand cases per year. The bubonic plague, resulting directly from infected fleas bite, is the more encountered plague form in Madagascar. Insecticide dusting is the method used to control rat fleas during epidemic season. In this study our two mains objectives are: evaluating the current status of flea population's susceptibility to insecticides and improving the methods allowing better control of fleas involved in plague transmission. Primarily, 12 insecticides, clustered in four families: organophosphate, carbamates, organochlorine and pyrethroid, were used to test the resistance level of *Xenopsylla cheopis*, the main plague vector. We found that *X. cheopis* from 30 out to 32 localities was found resistant to deltamethrin, a pyrethroid the first-line insecticide used to control plague during at least one decade. More, deltamethrin resistant fleas were resistant to nearly all tested insecticides, except dieldrin, an organochlorine. Besides, resistance level to each insecticide was different according to flea's origin, complicating the vector plague management. Thus, it becomes crucial to find more targeted approach to fight against rat fleas. One way which worth to be explored is the use of systemic insecticide, by incorporating insecticide in rodents baits. We assessed acute toxicity of fipronil, by contact and by the systemic way, on highly deltamethrin resistant fleas. No resistance to this compound was noticed at 0.05% when applied to fleas by contact, with 100% mortality. However, the toxicity against fleas was 90 fold higher when mixed to Rattus rattus and R. norvegicus baits. The use of systemic insecticide as a method for controlling vectors of *Y. pestis* has many benefits compared with insecticide dusting, by targeting rat fleas directly on their host, then reducing the cost and the amount of insecticide spilled in the environment.

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SOURCE OF HOST BLOOD AFFECTS LOCALIZATION OF THE BLOOD MEAL AND INFECTION PREVALENCE OF *YERSINIA PESTIS* IN THE FLEA VECTOR

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Flea-borne transmission of *Yersinia pestis,* the etiologic agent of plague, can occur during the first four days of infection by an unknown mechanism termed early-phase transmission (EPT). One week or later post-infection, *Y. pestis* is transmitted by the biofilm-dependent regurgitation mechanism. In laboratory studies of flea vector competence, infection rates and transmission efficiencies vary considerably between flea species as well as among conspecifics from different studies. One variable that may explain this variation is the species of animal blood used in the infectious blood meal. To investigate the effect of host blood source on flea vector competence, we determined the infection prevalence in groups of *Xenopsylla cheopis* rat fleas one day after they had fed on mouse, rat, or sheep blood containing equivalent concentrations of *Y. pestis*. In addition, we examined the flea digestive tract to determine the localization pattern of recently ingested blood. Consistent with previous studies, fleas fed on infected rodent blood had high infection rates (85-100%). In contrast, most fleas that fed on sheep blood cleared the infection (10-20% infection rate) within one day. Interestingly, 15-30% of fleas that fed on infected rat blood were observed to have blood in the esophagus (BIE), in addition to the midgut, within 24 hours after an infectious bloodmeal. In contrast, little to no BIE (≤1%) was observed in fleas that fed on sterile blood or fleas that fed on infected mouse or sheep blood. Upon feeding again, within three days of infection, a subset of fleas with BIE retained a combination of older blood and the developing mass of plague bacilli in the esophagus which appeared to obstruct passage of the most recent blood meal. Our results indicate that the source of host blood influences the likelihood that a flea will become persistently infected with *Y. pestis*. In addition, the presence of rat blood and bacteria in the flea foregut may impede feeding sufficiently to influence the efficiency of EPT and is suggestive of a regurgitative mechanism for this type of transmission.

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VECTOR COMPETENCY OF TICK-BORNE RELAPSING FEVER SPIROCHETES

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Tick-borne relapsing fever (RF) spirochetes are globally distributed pathogens that cause significant morbidity and mortality if left untreated. Most species are primarily transmitted by argasid ticks in the genus *Ornithodoros*. A defining characteristic between *Ornithodoros* species and RF spirochetes is vector specificity, where a given species of tick only transmits a specific species of spirochete. We have developed the *Borrelia turicatae-Ornithodoros* turicata model of RF spirochetes. The vector is distributed in the southern United States into Latin America. In the United States there is a western population of *O. turicata* ranging from California to Texas, a gap through Louisiana, Mississippi, and Alabama where the tick has not been identified, and an isolated eastern population in Florida. This eastern population has previously been designated a subspecies, *O. turicata americanus*. A current gap in knowledge is vector competency between western and eastern populations of *O. turicata* for geographic isolates of *B. turicatae*. In this study we established uninfected tick colonies of *O. turicata* that originated in Floirda, Texas, and Kansas. Vector competency and transmission studies were performed with the Oz (Ozona, Texas) and Florida canine *Borrelia* (Sumter County, Florida) isolates of *B. turicatae*. Furthermore, with salivary gland colonization essential for transmission of RF spirochetes, the tissues from each group of tick were assessed for infection by *B. turicatae* isolates. Our results indicate significant differences in the ability of Florida, Texas, and Kansas ticks to maintain and subsequently transmit geographically distinct *B. turicatae* isolates.

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RETROTRANSPOSON-TARGETED BLOODMEAL REMNANT IDENTIFICATION IDENTIFIES MEADOW VOLES AS THE MAIN HOST FOR SUBADULT DOG TICKS

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Martha's Vineyard, MA has continuously sustained endemic transmission of *Francisella tularensis* for over 15 years. Our study site comprises a stable natural focus that depends on dog ticks (*Dermacentor variabilis*). However, efforts to identify the *F. tularensis* reservoir have been hindered by low trap success and failure to detect evidence of infection from any sampled animals. It is likely that most infected hosts die rapidly of infection and are thus less likely to be trapped. We have previously reported on results of bloodmeal remnant identification analyses to determine which animals serve as the major host for subadult dog ticks in our site and thereby incriminate a potential reservoir. Assays described in the literature fail to amplify more than half of sampled ticks; we find them to be poorly sensitive, unreliable and difficult to reproduce. We report on an assay with greater sensitivity and reproducibility that will work with all ticks regardless

of extraction method. Retrotransposons integrate into genomes and replicate themselves; 40% of a mammalian genome can be made up of such "junk DNA", yielding more than a thousand copies of any particular one. In addition, retrotransposons have ancient origins and have coevolved with different families of mammals making it possible to design host-specific PCR primers. Accordingly, we designed real time retrotransposon PCR primers that can distinguish between the possible host species found on the island: mice, voles, rabbits, shrews, chipmunks/squirrels, deer, rats and raccoon/skunks. Archived dog tick DNA templates from 2006 were screened by real-time PCR, including samples that had previously failed to yield host bloodmeal remnant identification using existing protocols. Of the 137 ticks tested, all amplified with at least one set of primers. The majority of ticks, 75% (103) tested positive for vole DNA, 13% (18) for mouse, 10% (14) for deer, and 2 samples had multiple positives results. We conclude that voles are the primary host for immature dog ticks on Martha's Vineyard and likely critical hosts for the maintenance of *F. tularensis*.

1302

UPREGULATION OF SPHERICAL BODY PROTEIN 2 COPY 11 IN *BABESIA BOVIS* IS A SIGNATURE OF ATTENUATION

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Babesia bovis is the causative agent of bovine babesiosis, a tick-borne hemoparasitic disease that affects more than 500 million cattle annually worldwide. Currently, the only effective strategy to alleviate economic losses caused by this disease is the use of a live, attenuated vaccine which has some inherent risks. Better understanding of how attenuation is acquired and the identification of factors expressed in the vaccine strains that contribute to protective immunity, are essential for vaccine improvement. Our long-term goal is to understand the biological processes behind virulence loss and acquisition in *Babesia* so that a safe and effective subunit vaccine, incorporating all the necessary attenuation components can be developed. Our published work comparing genomes of three geographically distinct virulent parental and attenuated strain pairs illustrated no changes at the shared coding level among the phenotypic strains although all attenuated derivatives consist of significantly reduced genomic diversity. Our subsequent transcriptomic investigation of two of the strain pairs revealed that spherical body protein 2 truncated copy 11 (*sbp2t11*) transcript was differentially regulated. Thus, we hypothesized that *sbp2t11* is a *B. bovis* attenuation marker. Using additional virulent and attenuated strain pairs, we confirmed that *sbp2t11* is translated and that expression of SBP2t11 is significantly higher in the attenuated strains. Further analysis of SBP2t11 demonstrated proteolytic processing of the full length 30 kDa protein into a 17 KDa carboxyl terminal-derived fragment via a PEXEL-like domain, a cleave signal shared with *Plasmodium*, *Toxoplasma*, *Cryptosporidium* but not *Theileria*. To determine if upregulation of *sbp2t11* directly contributes to the attenuated phenotype, stable transfected virulent *B. bovis* over expressing *sbp2t11* was successfully generated. Ongoing *in vivo* experiments will determine if *sbp2t11* upregulation in virulent *B. bovis* recovers the attenuated phenotype.

1303

LARGE-SCALE DRUG SCREENING AGAINST *BABESIA DIVERGENS* PARASITE USING A FLUORESCENCE-BASED HIGH-THROUGHPUT SCREENING ASSAY

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Babesia divergens causes a serious infection in both humans and cattle in Europe. A severe *B. divergens* infection is a rapidly fatal disease showing clinical symptoms such as fever, malaise, hemolytic anemia, and end fatally with general organ failure 4–7 days after hemoglobinuria. Chloroquine was the initial pharmacologic agent thought to be effective against *B. divergens*. Diminazene aceturate, an antiprotozoal compound known to be effective in veterinary cases, failed to cure a patient with a severe *B. divergens* infection. Recently, antibabesial drugs commonly used in veterinary medicine have shown problems regarding parasitic resistance and toxicity to the host. Thus, the search for novel alternative compounds for the veterinary market is urgent. In this study, the validation of a fluorescence-based high-throughput screening (HTS) assay for determining the efficacies of large chemical libraries against *B. divergens* (bovine strain) in *in vitro* cultures is evaluated. Hematocrits (HCTs) of 2.5%, 5%, and 10% were used for the *in vitro* culture at 1% parasitemia without daily replacement of the medium. Linearity and HTS assay results revealed that the best HCTs were 5% and 10%. The obtained IC_{50} values of diminazene aceturate, either by fluorescence-based HTS assay with and without daily replacement of medium or by fluorescence- and microscopy-based methods, did not differ significantly at 5% HCT. Actinonin was the most effective drug against the *in vitro* growth of *B. divergens*, followed by diminazene aceturate and then chloroquine diphosphate, while moderate activity was observed with pyronaridine tetraphosphate- and luteolin-treated cultures. On the contrary, tetracycline hydrochloride and (-)-epigallocatechin-3-gallate from green tea exhibited poor activity as compared with diminazene aceturate (positive control drug). The data indicated that 5% HCT without daily replacement of the culture medium mixed with bovine serum *in vitro* using a fluorescence-based HTS assay creates the best conditions for large-scale drug screening against *B. divergens* that infect cattle.

1304

EVALUATION OF INHIBITORS OF *LEISHMANIA* PARASITOPHOROUS VACUOLE DEVELOPMENT

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Leishmania parasites are able to evade host immune responses during infection by residing within parasitophorous vacuoles (PVs), which are specialized phagocytic compartments formed within phagocytic immune cells during *Leishmania* infection. Our lab has previously shown that PVs contain molecules from both the secretory and endocytic pathways that are actively recruited by live parasites during infection. Disruption of this recruitment through the knockdown of secretory pathway vesicle fusion molecules such as Sec22b and Syntaxin-5 (Stx5) reduced the size of PVs that harbored *L. mexicana* parasites and inhibited parasite growth within Raw264.7 macrophages. In follow up studies we showed that Retro-2, a member of a novel class of small retrograde inhibitor molecules, specifically inhibits Stx5 localization, which results in reduced PV sizes and parasite numbers during *L. mexicana* infections. Moreover, Retro-2 reduced mouse foot pad lesions and parasite burden during *in vivo* infections of Balb/C mice. In addition, Retro-2 had a direct inhibitory effect on parasite replication in axenic culture. The purpose of this study

was to determine if secondary derivatives of Retro-2 are more effective at killing parasites and controlling parasite replication in axenic culture and during *in vitro* infections. We have found that the secondary derivative dihydroquinolinone 36 (DHQZ 36), kills L. amazonensis parasites with an IC50 of 10μM, which demonstrates that it is more potent at killing *Leishmania* parasites than the parent drug. In light of the fact that this class of drugs targets the secretory pathway, specifically Stx5 and in light of the fact that *Leishmania* parasites have been shown to have a limited number of secretory pathway SNARE genes, we evaluated the effect of DHQZ 36 on parasite secretion. We will show that prior to killing *Leishmania* parasites, DHQZ 36 limits secretion from by the parasite. This effect on parasite secretion by this new class of molecules has important implications for its function within macrophages where parasite secretion appears to play important roles in the parasite-induced evasion mechanisms.

1305

EVALUATING PKB/AKT AS THE TARGET OF MILTEFOSINE IN *LEISHMANIA* TREATMENT

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Miltefosine is an orally administered drug that is currently used to treat *Leishmania* infections. In light of the fact that some *Leishmania*s exhibit inherent resistance to Miltefosine and that there is increasing concern of the emergence miltefosine-resistant *Leishmania* strains, there is urgency to identify miltefosine's target and the mechanism of miltefosine resistance. Our previous studies had shown that *Leishmania* infection of macrophages induces sustained activation of PKB/AKT, a downstream kinase in the PI3K signaling pathway. In light of the fact that miltefosine was originally shown to target PKB/AKT in tumor cells we set out to test the hypothesis that PKB/AKT is the target of miltefosine in *Leishmania* infections. To evaluate whether PKB/AKT is the target of miltefosine, we have generated macrophages that express inducible shRNAs specific for AKT1 by transduction of a single lentivirus vector constructed to express oligos that target the AKT1 gene. Control cells expressing either the lentivirus alone or lentiviruses expressing PGK were generated as well. After infecting with *Leishmania* parasites, suppression of PKB/AKT levels was induced by adding doxycycline which induced shAKT1 production. Even prior to adding Miltefosine, reduction of PKB/AKT levels in cells resulted in death of 40% of parasites within infected cells. Remarkably, Miltefosine even at relatively high concentrations was unable to reduce the number of parasites further. To further assess PKB/AKT as a target for killing *Leishmania* parasites, we evaluated other specific inhibitors of PKB/AKT. Cells in which the AKT1 specific shRNA was induced were refractory to killing by the specific inhibitors of PKB/AKT. These parasites could be killed with paramomycin, whose mechanism of action is known not to include PKB/AKT signaling. Taken together, these results implicate PKB/AKT as the target of miltefosine. We are presently evaluating whether the capacity to induce activation of PKB/AKT by *Leishmania* parasites correlates with their relative resistance or susceptibility to miltefosine.

1306

THE COMPOSITION OF MICROVESICLES DERIVED FROM *LEISHMANIA DONOVANI* INFECTED MACROPHAGES PROVIDES PERSPECTIVES INTO THEIR BIOGENESIS AND CONTRIBUTIONS TO PARASITE PATHOGENESIS

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Extracellular microvesicles have emerged as important mediators of cell-to-cell communication and have been shown to contribute to the pathogenesis of microorganisms. To better understand the properties of extracellular vesicles produced by *Leishmania donovani* infected macrophages, we performed comparative proteomics of microvesicles derived from RAW 264.7 mouse macrophages uninfected or infected with *L. donovani*. We have obtained a preliminary profile of the host and parasite derived proteins in the microvesicles released from infected and uninfected macrophages. In addition to host cell derived molecules previously identified by others in exosome preparations, we have observed in microvesicle preparations obtained from infected cell cultures significant representation of the exocyst complex component 3 (Exoc3), which has been implicated as a mediator of the release of some microvesicles. In additional analyses we have compared the proteomic profiles obtained from these experiments with recently published studies of the composition of exosomes released by axenically cultured *L. donovani*. The putative 40S ribosomal protein S3a and RPL3 found in exosomes from axenically grown parasites were not found in the microvesicles from *Leishmania*-infected macrophages. Conversely, a putative condensin subunit 1, phosphatidylinositol 3-kinase, and signal recognition particle molecules were found in microvesicles from *Leishmania*-infected macrophages but were not found in exosomes from axenically grown parasites. To confirm the identification of *Leishmania* derived molecules, comparable preparations were obtained from infections with parasites that lack the centrin gene, making these parasites unable to mature into the intracellular amastigote form. Our results show a significant diminution of the parasite derived molecules from macrophages derived from centrin knockouts. Taken together, we will present a comprehensive molecular profile of the molecules released in extracellular microvesicles from *L. donovani*-infected macrophages. Insight in the mechanism of their biogenesis will be presented as well.

1307

CHARACTERIZATION OF THE TRYPANOSOMATID SECONDARY ALTERNATIVE OXIDASE - A NOVEL POTENTIAL DRUG TARGET

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Kinetoplastid parasites of the *Trypanosoma* and *Leishmania* genera cause widespread disease and death in much of the developing world. Current treatments are outdated, increasingly ineffective and associated with severe adverse effects, and new therapies are urgently needed. One of the limiting factors in the drug discovery pipeline is the identification of useful drug targets. The trypanosome alternative oxidase (TAO) has been well characterized as a drug target in *T. brucei*, but is absent from *T. cruzi* and *Leishmania* spp. Here we report evidence of a previously uninvestigated secondary alternative oxidase (AOX2) that is expressed in all three parasites, but importantly has no mammalian ortholog, making it an attractive drug target. Using reverse genetics we have shown that AOX2 is an essential protein in *L. major*, *T. cruzi* and bloodstream form *T. brucei*. By overexpressing AOX2 *in vivo* we have confirmed the subcellular localization of AOX2 to be mitochondrial, as it is for TAO in *T. brucei*. We are examining the effects of AOX2 overexpression/underexpression on cell growth and mitochondrial respiration in *T. brucei* and *T. cruzi* to determine the role of this protein in these parasites. We have established optimal conditions for recombinant expression of the *T. brucei*, *T. cruzi* and *L. major* AOX2 in *E. coli*. We have solubilized and purified all three AOX2s allowing enzyme activity studies. We are screening for selective inhibitors of these AOX2s using our in-house natural product-like library and a fragment library to identify lead compounds. Therefore we are genetically and chemically validating AOX2 in trypanosomatids.

1308

UNDERSTANDING THE ROLE OF *LEISHMANIA* RNA VIRUS-1 (LRV-1) IN THE PATHOGENESIS OF AMERICAN TEGUMENTARY *LEISHMANIA*SIS USING A HUMAN MACROPHAGE MODEL

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Leishmania RNA virus-1 (LRV-1) is a double stranded RNA virus primarily identified in the *Leishmania Viannia* complex endemic to Latin America. LRV-1 has been documented in 20-25% of *Leishmania Viannia* guyanensis and *L. V. braziliensis* strains, known to progress to mucocutaneous leishmaniasis, found in Brazil and Peru, and has been been correlated to increased levels of proinflammatory cytokines and chemokines. Our objective was to compare biomarker expression in human macrophages infected with LRV1-positive or negative strains of *Leishmania*. Human monocytes (U937) were transformed to macrophages over 72 hrs at 37 C. Promastigotes of LRV-1 positive (*L. V. guyanensis*, *L. V. braziliensis*) and LRV-1 negative (*L.V. braziliensis*, *L. tropica, L. infantum*) strains of *Leishmania* were inoculated into macrophage cultures, and culture supernatants were obtained at 24-, 48-, and 72-hrs. Proinflammatory markers measured by ELISA at 24-, 48-, and 72-hrs of incubation included IL-1β, IL-4, IL-5, IL-6, IL-12, TNF-α, CXCL10, CCL5, iNOS, and superoxide dismutase (SOD). Virulence factor transcript expression, including Heat Shock Protein 20 (HSP20), HSP70, HSP83, Mannose Phosphate Isomerase (MPI), Cysteine Proteinase B (CPB), zinc-metalloproteinase (GP63) was quantified in by real time RT-PCR. LRV-1 status did not affect expression of TNF-α, CXCL10, or IL-6, however, LRV-1 positive strains had increased CCL5 (p=0.022) and reduced IL-1 (p=0.023) expression at 24- and 48-hours, respectively. SOD expression was reduced in all macrophages infected with *Leishmania* spp. regardless of LRV-1 status. The LRV-1 positive *L. V. braziliensis* strain showed higher levels of HSP20, HSP83 and GP63 expression compared to the LRV-1 negative *L. V. braziliensis* strain with 41-, 14- and 92% increases, respectively. LRV-1 status remains a potential biomarker of disease severity in American tegumentary leishmaniasis. Our observation of varying cytokine, chemokine, and virulence factor expression by LRV1 status suggests that LRV-1 could potentially contribute to the mechanism by which *L. V. braziliensis*, in particular, leads to pathogenicity.

1308

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1309

INVESTIGATING VIRUS PERSISTENCE IN BODY FLUIDS OF EBOLA SURVIVORS IN SIERRA LEONE

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At the start of the West African Ebola epidemic, there was limited evidence on persistence and duration of Ebola virus (EBOV) and viral ribonucleic acid (RNA) in semen or other body fluids of Ebola Virus Disease (EVD) survivors. The Sierra Leone Ebola Virus Persistence Study (VPS) is an observational cohort that aims to assess the presence and duration of EBOV and viral RNA in semen and other body fluids of EVD survivors. A pilot study launched in May 2015, which enrolled 100 male EVD survivors in the Western area, collecting and testing semen biweekly until two consecutive qRT-PCR negative results were obtained. The main study launched in November 2015, enrolling 120 male and 120 female EVD survivors primarily from Western and Port Loko districts. Sweat, saliva, tears, urine, rectal swab, and as appropriate semen or vaginal swab, menstrual blood, and breast milk were collected. Virus isolation was attempted on qRT-PCR positive specimens. Participants from the pilot and main studies received qRT-PCR test results and risk reduction counseling. To date, the longest period of EBOV RNA detected in semen of pilot participants was 406 days post-onset of symptoms. Proportions of participants with qRT-PCR positive semen decreased with increasing time post-onset. Four semen specimens yielded EBOV isolates; the longest period of time post-onset that viable EBOV was detected was 157 days. To date, 84 women and 92 men have enrolled in the main study. For males and females tested at 6 to 19 months post-onset, all body fluids were qRT-PCR negative, with the exception of 15 men who had EBOV RNA detected in semen and a single male participant who had qRT-PCR positive urine detected to 292 days post onset, during which time his semen also tested positive. A female participant enrolled in the study on the day of discharge from an Ebola Treatment Unit had qRT-PCR positive vaginal swab specimens until day 35 post-onset. All other body fluids tested in this woman were negative. These preliminary descriptive results provide valuable information regarding EBOV persistence in EVD survivors long after recovery.

1310

MAPPING ANTIBODY EPITOPES ON THE EBOLA VIRUS ENVELOPE PROTEIN BY SHOTGUN MUTAGENESIS

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To characterize the detailed immune response to Ebola virus (EBOV), we applied high throughput epitope mapping to the EBOV envelope glycoprotein (GP). We used Shotgun Mutagenesis to create a comprehensive alanine scan library comprising 641 single mutations in GP individually arrayed in 384 well plates. GP variants were expressed in human cells and assayed for reactivity with monoclonal antibodies (MAbs) using high-throughput flow cytometry to identify GP residues required for the binding of each MAb. Cocktails of MAbs that target EBOV GP have great promise as therapeutics. However, for ZMapp, the most advanced cocktail, the detailed epitopes are not known. We epitope mapped the ZMapp and related ZMAb and MB-003 cocktails, resolving the amino acid epitopes for all six MAbs in these cocktails and for the standard reference MAb KZ52. We have also epitope mapped over 90 additional MAbs that recognize EBOV GP, including 6 MAbs obtained from a human survivor of Bundibugyo ebolavirus infection, and a MAb, cross-reactive with ebolavirus species, that binds to GP1 head and blocks the interaction of EBOV GP with its endosomal receptor Niemann-Pick C1. Identification of epitope residues for the ZMapp MAbs helps to: distinguish between MAbs that bind competitively in the same GP region but use different epitope residues, explain their reactivity against different EBOV species, predict viral evasion against these MAbs, and design new cocktails of MAbs that may offer improved functional complementarity. Epitope mapping is expanding our understanding of how the immune system recognizes EBOV GP. Correlating MAb epitopes with their neutralizing capabilities is being used to develop anti-EBOV therapeutics and vaccines.

1311

IDENTIFICATION OF HUMAN T CELL EPITOPES IN THE EBOLAVIRUS GLYCOPROTEIN FOLLOWING VACCINATION WITH CHAD3 EBO Z GP AND MVA BN

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Despite research into the immune response to Ebolavirus for several years, no human CD8+ T cell epitopes have been described following either natural infection or vaccination with any of the candidate vaccines. Here we present several novel 9 amino acid-long peptide epitopes in the Zaire Ebolavirus glycoprotein from humans vaccinated with the ChAd3-MVA regime. Primary studies of T cell immunogenicity were performed using IFNγ ELISPOT assays with ten pools of overlapping peptides 13-17 amino acids long on samples from 43 volunteers that had received a priming immunisation of ChAd3 EBO Z GP and a booster immunisation with MVA BN Filo between 3 and 10 weeks later. We then analysed seven pools in detail using individual 15mer peptides to select the most immunodominant sequences in a subset of volunteers. Overlapping 9mer peptides were then synthesised spanning the 18 dominant 15mers in these pools and assayed by ELISPOT in the same samples. The responses to epitopes were further categorised with intracellular cytokine staining to show large production of IFNγ and TNFa in the CD8+ cytotoxic T cell lineage. The epitopes described span the length of the GP protein and were found in both the GP1 and GP2 domains. We also performed HLA typing on these volunteers and demonstrate broad recognition of these epitopes on multiple HLA backgrounds, suggesting that this vaccine is likely to be immunogenic in genetically diverse populations. Analysis of the position of the epitopes within the 3-dimensional structure of the protein is ongoing and will be presented. The novel human T cell epitopes discovered could be utilised for future vaccine development, as well as in further understanding the immune response to the ChAd3-MVA vaccine and EBOV infection.

1312

THE SIERRA LEONE TRIAL TO INTRODUCE A VACCINE AGAINST EBOLA (STRIVE)

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In response to the 2014-16 Ebola epidemic, clinical development of candidate Ebola vaccines was accelerated. By late 2014, Phase 1 studies of candidate vaccines started, and multiple organizations began planning phase 2/3 studies with collaborators in the most heavily Ebola-affected countries. The US Centers for Disease Control and Prevention sponsored a phase 2/3 vaccine trial In Sierra Leone, in collaboration with the College of Medicine and Allied Health Sciences, University of Sierra Leone and the Ministry of Health and Sanitation. STRIVE was designed as an individually randomized trial for phased introduction of a single 2 x 107 pfu/mL dose of candidate rVSVΔG-ZEBOV-GP vaccine in healthcare and frontline Ebola response workers, while simultaneously evaluating vaccine safety and efficacy. No placebo was used. Participants were randomized to immediate (≤7 days) or delayed (18-24 weeks) vaccination and followed for 6 months after vaccination for serious adverse events (SAE) and Ebola virus disease (EVD). Reactogenicity data were collected through a safety sub-study of the first ~400 participants (200 vaccinated, 200 unvaccinated). An immunogenicity sub-study of ~500 participants assessed IgG levels at baseline, 28 days, 6 months, and 9-12 months after vaccination using a glycoprotein Elisa assay. Enrollment began on April 9th, and vaccination ended December 12th 2015 with ~8,650 participants enrolled and ~8,000 vaccinated. As of 1 April 2016, preliminary data from ongoing safety follow up indicates no vaccine-related deaths or other vaccine-related SAEs; 48 participants were evaluated for EVD and had negative test results. Systemic symptoms more commonly reported in vaccinated safety sub-study participants included headache, fever/feverishness, fatigue, muscle pain and joint pain; few were graded as severe. Ebola response measures successfully interrupted transmission, so vaccine efficacy could not be assessed. STRIVE provides the largest SAE database on this vaccine and will yield critical immunogenicity data to support a vaccine licensure application. Preliminary immunogenicity data will also be presented.

1313

SAFETY, IMMUNOGENICITY, AND EFFICACY OF THE MERCK RVSVΔG-ZEBOV-GP EBOLA VACCINE

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The 2013-2016 Ebola outbreak has caused over 28,000 cases and 11,000 deaths. Merck & Co., Inc. is working with private and public partners to develop an Ebola vaccine that has demonstrated efficacy during this outbreak. The vaccine is a live recombinant vesicular stomatitis virus (VSV) with complete substitution of the VSV-G envelope glycoprotein (GP) with

Zaire ebolavirus GP. Phase 1-3 clinical trials have been conducted to assess safety, immunogenicity, and/or efficacy of rVSVΔG-ZEBOV-GP in humans. One intramuscular dose of $2x10^7$ plaque forming units is well-tolerated when administered to healthy adults. Injection site reactions following vaccination are typically mild or moderate and self-limited. There is a predictable period of generally mild reactogenicity, including fever and a flu-like syndrome typically lasting 1-3 days. Joint pain is a common part of the early flu-like syndrome. In a small proportion of subjects (<5% in most studies), joint swelling (arthritis) may develop in the weeks following vaccination. Arthritis is generally mild to moderate in severity and is likely mediated by direct viral infection of joint tissues; the vast majority of arthritis events resolve spontaneously, though persistent and recurrent symptoms have also been reported. Anti-GP antibodies are detectable by ELISA by 14 days postvaccination in 95% of vaccinees; to date, 100% seroconversion has been observed by 28 days. Durability of the anti-GP response response has also been demonstrated for at least 6 months. A ring vaccination trial in Guinea randomized 7,651 subjects in 90 clusters to receive immediate or delayed vaccination. Interim analysis identified no cases of Ebola virus disease with symptom onset at least 10 days after randomization in the immediate vaccination group, whereas in the delayed vaccination group there were 16 cases of Ebola virus disease (vaccine efficacy 100%, 95% CI: 74.7, 100.0; p=0.0036). No new cases of Ebola virus disease were diagnosed in vaccinees from the immediate or delayed groups from day 6 postvaccination. In this presentation we will provide the current status of the rVSVΔG-ZEBOV-GP development program.

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PHASE 1 EVALUATION OF A LIVE ATTENUATED HUMAN PARAINFLUENZA VIRUS TYPE 3 VECTORED VACCINE CANDIDATE EXPRESSING EBOLAVIRUS ZAIRE GLYCOPROTEIN

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The 2013 outbreak of Ebola Virus Disease in West Africa brought into sharp focus the urgent need for safe and effective medical countermeasures. The primary objectives of this open label vaccine clinical trial were to determine the safety, tolerability and immunogenicity of a 2-dose regimen of a live, recombinant parainfluenza virus type 3 expressing the Ebola virus (Zaire) glycoprotein (HPIV3-EbovZ-GP) as a vaccine candidate in healthy adults. Thirty subjects were enrolled sequentially into 2 cohorts of 10 and 20 subjects, respectively. The investigational new drug (IND)-approved protocol plan was for both cohorts to receive 2 doses of the vaccine candidate intranasally 4 to 8 weeks apart as follows: Cohort 1 to receive 10^6 plaque forming units (PFU) of HPIV3-EbovZ-GP vaccine and Cohort 2 to receive 10^7 PFU. During protocol implementation, two doses of 10^6 PFU of HPIV3-EbovZ-GP vaccine were administered to Cohort 1 subjects 4 weeks apart. The vaccine was well tolerated and infectious (7/10 subjects had detectable virus by rRT-PCR with a mean peak titer 3.8 $\log_{10}$ genomic equivalents/mL and 4/10 with vaccine virus on culture.) The viral shedding period was longer than expected with a mean of 7.9 days after vaccination. Little shedding was detected after the second dose. Cohort 2 received one of two planned doses of 10^7 PFU of HPIV3-EbovZ-GP vaccine. Vaccine virus infectiousness in Cohort 2 was similar to Cohort 1 but shorter in duration, with a mean of 3.7 days of shedding. In both Cohorts, the vaccine was well tolerated; the majority of symptoms mild. Asymptomatic ALT elevations were noted in 5 volunteers in Cohort 2 after vaccination: 3 mild elevations, 2 moderate (68-184 U/L). ALT elevations were associated with viral shedding, all had resolved by day 28. The study was halted due to these elevations of ALTs and the volunteers in Cohort 2 were not given the second dose. Expression of Ebola virus GP was stable in the virus shed by volunteers. Little to no detectable neutralizing antibody was induced in the subjects. We conclude that the HPIV3-EbovZ-GP vaccine virus is more infectious but less immunogenic than anticipated with longer viral shedding periods.

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SEROPREVALENCE OF FILOVIRUS INFECTION IN VILLAGES WITH NO HISTORY OF OUTBREAK IN THE DEMOCRATIC REPUBLIC OF CONGO

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The Democratic Republic of Congo (DRC) has experienced the most Ebola Virus Disease (EVD) outbreaks on record. While evidence is limited, previous studies suggest that there may be a significant number of cases of Ebola and other viral hemorrhagic fevers (VHFs) that go unreported because they are non-progressing or asymptomatic. In 2007, a population-based survey was conducted to assess human exposure to monkeypox in healthy, rural populations in the Kole and Lomela health zones within the Kasai Oriental province of DRC. Fourteen villages were randomly selected and all healthy individuals ≥1 year of age were eligible. Among the 5,687 individuals eligible for enrollment, 4,574 were enrolled in the original study population, of whom a subset (n=810) were randomly selected for serologic assessment of antibody response to Ebola (EBOV) and Marburg (MARV) viruses. In this preliminary study, nearly all subjects (>99%) were from Lomela health zone. Overall, 7% of subjects tested positive for EBOV in either neutralizing assay (confirmed with titration assay), viral matrix protein (VP40) ELISA or nucleoprotein (NP) ELISA, while 2% tested positive for MARV in either neutralizing assay (confirmed with titration assay) or nucleoprotein (NP) ELISA. Prevalence of filovirus antibody varied slightly by age; specifically 1-4 year olds had the highest prevalence for both EBOV and MARV (11% and 3%, respectively). Additionally, seropositivity was significantly associated with gender; EBOV seropositivity was higher in males than females (5% and 9%, respectively), while MARV seropositivity was higher in females than males (3% and 1%, respectively). While analyses are preliminary, we found evidence of infection in vulnerable subgroups of the population in non-outbreak locations in the DRC. Such sero-surveillance studies are of paramount importance as they may provide key information about asymptomatic or non-progressing infections that can better prepare health care workers and policy makers for future outbreaks.

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IMMUNOASSAYS FOR CHARACTERIZING AND EVALUATING VACCINE CANDIDATES THAT TARGET PRE-ERYTHROCYTIC STAGES OF *PLASMODIUM VIVAX* AND *P. FALCIPARUM*

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Evaluating pre-erythrocytic (PE) vaccine candidates is challenging due to the biological complexity of *Plasmodium* parasites and their host interactions. In particular, identifying and characterizing new PE vaccine candidates has been hampered by the inefficiency of functional assays to assess targets in the PE stages of *P. vivax* and *P. falciparum*. To overcome this obstacle, we developed novel functional assays to immunologically assess potential PE targets of *Plasmodium* sporozoites and early liver stages. The functional assays recapitulate the pivotal period of sporozoite transition from mosquito to human by physical modification of the *in vitro* culture microenvironment and exposure to biological stimulatory factors. In this study, liver-stage development was assessed by sporozoite inoculation into an *in vitro* human liver model platform where high content image analysis was used to quantify parasite invasion and initial development in primary human hepatocytes. As proof of concept for assessing PE vaccine candidates, inhibition of liver-stage development assays (ILSDA) targeted the *P. vivax* and *P. falciparum* circumsporozoite (CSP) protein with species-specific, anti-CSP monoclonal antibodies. The modified ILSDA was sensitive and efficient, showing blocking of early PE stages of both *P. vivax* and *P. falciparum* in a concentration-dependent manner. Further ILSDA experiments were conducted with a second target antigen, which showed nanomolar activity with 80% inhibition of liver-stage development.
To refine functional analysis of PE targets in early infection phases, we analyzed sporozoite migration in real-time in a 'live' motility assay and a hepatocyte cell traversal assay, and also by high-content imaging. Finally, micro-pillar arrays were designed for real-time study of sporozoite migration and mechanical flexibility in a structured microenvironment mimicking *in vivo* conditions. Together, these novel functional assays simulate key development and transition phases that enable us to evaluate potential PE vaccine candidates and analyze complex *Plasmodium* sporozoite phenotypes.

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GAMETOCYTE-SPECIFIC IMMUNITY PROVIDES A RATIONALE FOR NOVEL TRANSMISSION BLOCKING INTERVENTIONS IN *PLASMODIUM FALCIPARUM*

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Drugs and vaccines targeting *Plasmodium falciparum* transmission stages have recently gained prominence as necessary tools for malaria elimination. Though most current transmission-blocking approaches focus on mosquito stages, targeting gametocytes - the form developing in the human host - provides a more convenient endpoint for validation, and has tremendous potential to reduce global transmission of malaria. Antibodies recognizing immature (stage I-IV) gametocytes could confer protection by 1) inhibiting binding interactions between specific host receptors and adhesins on the surface of infected red blood cells (iRBCs), 2) increasing killing by effector cells, and/or 3) inducing uptake by other immune cells. Hypothesizing that early-stage gametocytes are targets of host antibody responses, we performed the first systematic characterization of immune responses recognizing these stages. Utilizing a gametocyte-enriched protein array, we identified a subset of exported parasite antigens whose reactivity correlates with exposure and/or reduced parasite burden. We next characterized recognition of the gametocyte-iRBC (giRBC) surface in a cohort of Malawian sera by flow cytometry. A subset of sera recognize both giRBCs and asexual-iRBCs (aiRBCs) while others uniquely recognize giRBCs. The strength and prevalence of both aiRBC and giRBC surface recognition increase with age, though this increase occurs more slowly for gametocytes. Immunofluorescence microscopy confirms that early (stage I-IIA) gametocytes are recognized more than later gametocytes, and Western blots using giRBC and aiRBC membranes provide evidence for both shared aiRBC-giRBC and unique giRBC antigens. Candidate antigens have been identified by mass spectrometry and their surface expression is being validated through various methodologies. We are also investigating various mechanisms of antibody-mediated protection, including opsonization for phagocytosis, and inhibition of binding to host receptors. Our study suggests a new paradigm for transmission stage immunity and provides a rational basis for novel transmission-blocking therapeutics.

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REPEATED MALARIA INFECTIONS ACCELERATES BIOLOGICAL AGEING IN CHILDREN IN DISEASE ENDEMIC AREA

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Accelerated ageing and reduced lifespan mediated through faster telomere shortening has recently been shown in birds chronically infected with malaria parasites. Furthermore, we have found that single malaria infection accelerates telomere degradation for up to three months after cured infection in human. Here, we have analyzed whether multiple malaria episodes accelerate telomere attrition in disease endemic area. Children living in Nyamisati village in the Rufiji river delta, coastal Tanzania were followed with passive case detection and repeated cross-sectional surveys between 1993 and 2010. Parasite prevalence in children declined from 90% in 1993 to 10% in 2010. Telomere length was analysed in peripheral blood by real time quantitative PCR in repeated cross-sectional surveys 1993-2010. Our preliminary findings show that telomere shortening was more pronounced in the children during the 1990s when transmission was moderate to high, compare to between 1999 and 2010

when transmission had declined. Children those were malaria positive in surveys have shorter telomere length and antimalarial antibodies levels were negatively correlated with telomere length, irrespective of age. These findings suggest that repeated malaria infections accelerate telomere shortening and might contribute to biological ageing in human. These results further urge the need for upscaling efforts to eliminate malaria.

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ATYPICAL ACTIVATION OF HUMAN PRIMARY DENDRITIC CELLS BY *PLASMODIUM FALCIPARUM*

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Malaria is characterized by high levels of inflammation and while an early inflammatory response is important for parasite clearance, excessive and persistent inflammation can contribute to severe forms of the disease. At the same time, *P. falciparum* infections fail to induce sterile immunity. Reports about the role of dendritic cells (DCs) in the immune response to *P. falciparum* and how they contribute to the activation of $CD4^+$ T cells during blood stage have been contradictory. To analyze this critical part of the malaria immune response we enriched primary human DCs from peripheral blood of naïve donors and co-incubated them with *P. falciparum*-infected red blood cells *in vitro*. Although DCs up-regulated surface expression of HLA-DR, co-stimulatory markers, and secretion of chemokines, they did not secrete significant amounts of inflammatory cytokines. Surprisingly, these parasite-activated DCs were able to activate and polarize naïve autologous $CD4^+$ T cells into Th1-like cells secreting high levels of IFNγ and TNF. A re-stimulation with autologous *P. falciparum*-activated DCs specifically increased proliferation and cytokine secretion of the primed $CD4^+$ T cells, indicating that the DCs were able to induce an antigen-specific response. We further analyzed the activation phenotype of the two major DC subsets, myeloid and plasmacytoid DCs, upon stimulation with *P. falciparum*. Co-culture of both subsets was essential to up-regulate parasite-induced chemokine secretion. Although plasmacytoid DCs were activated by the parasite through TLR9 and secreted IFNα, they were dispensable for $CD4^+$ T cell activation. To further address whether this phenotype might involve activation molecules other than MHC class II we analyzed CD1-restricted T cell activation. Preliminary results indicate that a proportion of $CD4^+$ T cells that expand in co-cultures recognize lipids presented by CD1. Our findings might contribute to a better understanding of the mechanisms involved in the initiation of the adaptive immune response against *P. falciparum*.

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ATYPICAL MEMORY B CELLS AND CIRCULATING MARGINAL ZONE-LIKE B CELLS CHANGES ASSOCIATED TO MALARIA CHRONIC EXPOSURE

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Malaria exposure affects circulating B and T cell populations, inducing increased frequency of "atypical" memory B cells (MBCs) and reduced proportion of circulating marginal zone (MZ)-like B cells. Although the role of antibody responses in reducing clinical symptoms is well known, little is known about the role of atypical MBC and MZ-like B cells against malaria infection. We characterized the status and function of B cell subpopulations in a malaria-exposed and unexposed populations and tested the hypothesis that impaired immune response was associated with anergic stage of B cells related to Pf exposure. We selected 45 adults from high malaria transmission area in Papua Nueva Guinea and classified them as high, medium and low exposure based on responses to 8 *Plasmodium falciparum* and *P. vivax* antigens. Peripheral blood mononuclear cells were assayed by flow cytometry to identify expression of activation-, inhibition-, lineage- and survival-associated markers. In exposed individuals, active atypical MBCs (aaMBCs) had high frequency of IgG, PD1, CD95, CCR3 and CD71 and low proportion of CD62L expression. The expression of PD1, CD95 and CD71 on aaMBCs was associated with level of exposure. As a result of chronic antigen exposure, aaMBCs have dual expression of both of CD40-CD95, leading aaMBC to an anergic state and at the same time preventing cell death. We found higher IgG- and PD1-expressing peripheral MZ-like B cells in malaria exposed compared to non-exposed adults. Conversely, TACI expression was greatly reduced in exposed individuals. Our findings suggest that in chronically exposed adults, the expression of PD1, CCR3 and CD95 on aaMBCs could be the result of immune homeostatic mechanisim for mantaining B cell development and function while simultaneously inhibiting hyper-reactive B cells. Thus, keeping B-cell activation threshold high enough to control but impaired enough to tolerate chronic infection. Increased IgG- and PD1- and decreased TACI-expressing MZ-like B cells confirm the role of germinal center-independent responses in chronically exposed individuals, being a potential target for reversion in anti-malarial therapies.

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KENYAN CHILDREN AND ADULTS WITH ACUTE UNCOMPLICATED MALARIA HAVE DYSFUNCTIONAL MEMORY B CELL RECALL RESPONSES TO POLYCLONAL STIMULATION

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The development of acquired immunity to *Plasmodium falciparum* (Pf) is not fully understood. It takes years of repeated exposure to Pf before immunity to clinical disease develops, implying that B cell memory is impaired and incomplete. B cells are a heterogeneous cell population, consisting of subtypes differentiated by their surface markers (naïve B cells $CD19^+CD21^+CD27^-$, classical memory B cells (MBC) $CD19^+CD21^+CD27^+$, activated B cells $CD19^+CD21^-CD27^+$, and atypical MBC $CD19^+CD21^-CD27^-$). ELISPOT assays were used to measure immunoglobulin (Ig) G secretion in peripheral blood mononuclear cells (PBMC) from Kenyan children aged 1-10 years at presentation of acute uncomplicated malaria and six weeks following treatment. Quantities of antibody secreting cells (ASC) were greatly reduced in children with active malaria, and restored 6 weeks following treatment (average numbers of ASC per 5000 plated PBMC were 6.67 and 52.67, respectively, p=0.0039). We also found that PBMC from an adult with acute malaria showed a similar outcome (4 ASC per 5000 PBMC during acute malaria versus 28 ASC six weeks following treatment). This suggests that there is a deficiency in the MBC recall response during acute illness, regardless of patient age. Flow cytometry was performed on PBMC in order to compare B cell subtype frequencies. Children with acute malaria and at 6 weeks follow-up had very similar frequencies of circulating classical MBC (average frequencies 14.49% and 17.56%, respectively, p=0.0419), which indicates the MBC were present in circulation at the time of acute malaria, though not functioning similarly. Interestingly, activated B cells were not increased at the time of acute malaria (average frequencies 4.98% and 3.42%, respectively, p=0.2663), while the acute cases showed some increase in proportions of atypical MBC during acute malaria versus recovery (average frequencies were 26.53% and 12.3%, respectively, p=0.0007). Future experiments will focus on determining if B cells or T cells play key roles in the lack of IgG secretion during acute malaria.

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CYNOMOLGI MALARIA IN RHESUS MACAQUES INDUCES PHENOTYPIC AND FUNCTIONAL CHANGES IN NEUTROPHILS

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The innate immune system likely plays a key role in *Plasmodium vivax* infection, but studies focusing on its impact have been limited. Neutrophils are the most abundant innate immune subset in blood and serve as a primary defense against microorganisms via phagocytosis, as well as the production and release of inflammatory mediators that amplify immune responses. However, excessive neutrophilic responses can lead to tissue damage and impact subsequent adaptive immune responses negatively. Thus, more detailed study of neutrophilic responses during malaria are warranted. In the context of *P. vivax* infection, previous studies have reported significant neutrophil activation, although more studies are needed to dissect out their diverse and dynamic functions. Here, the rhesus macaque – *P. cynomolgi* model was used as a model for vivax malaria to assess functional changes in neutrophils during infection. Five rhesus were infected with *P. cynomolgi* and their neutrophil function assessed during acute infection using flow cytometry-based assays. Neutrophils became activated based on expression of surface markers such as CD63 during infection, demonstrating a role for this cellular subset during *P. cynomolgi* infection. Additionally, neutrophil caspase-1 activity, measured by the fluorescent substrate FLICA, increased and correlated with an increase in IL-1β in the plasma. By contrast, neutrophils were impaired in both phagocytic ability and reactive oxygen species production during acute infection. Changes in neutrophil function correlated with parasitemia. Following these pilot data, our group is now examining the role of the various circulating neutrophil subsets (e.g., bands, segmented, and hypersegmented) to determine if a relationship exists between the functional changes observed during cynomolgi malaria and the abundance of such subsets. Indeed, humans with vivax malaria display changes in the frequency of neutrophil subsets in the blood during infection, which emphasize the relevance of our *P. cynomolgi* model. Overall, this model will be useful for better understanding neutrophil dynamics and potential functions during malaria.

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SPECTRUM-MALARIA: A USER-FRIENDLY PROJECTION TOOL FOR HEALTH IMPACT ASSESSMENT AND STRATEGIC PLANNING FOR MALARIA PROGRAMS IN SUB-SAHARAN AFRICA

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The Spectrum suite of policy planning models is used by over 120 countries to support estimation of burdens, trends, service needs and program impact. We developed the Spectrum-Malaria module, that projects impact of malaria interventions on case incidence and deaths in 0-4 year, 5-14 year and 15+ year olds, and falciparum infection prevalence (PfPR) among children 2-9 years, based on user-specified targets for people sleeping under insecticide-treated nets (ITNs), people protected by indoor residual spraying (IRS), children 6-59 months old receiving 3 courses of seasonal malaria chemoprophylaxis, and uncomplicated and severe cases effectively managed. Intervention effectiveness was estimated by generalized linear statistical models that emulate impact drivers as simulated by the dynamic OpenMalaria model. ITN and IRS effectiveness were validated on child health outcomes in 3 ITN trials. Spectrum projects impacts at province level starting from 2015 case, death and coverage levels, PfPR and seasonality estimated by the Malaria Atlas Project and World Health Organization. Pilots for Democratic Republic of the Congo (DRC) and Zambia, Nigeria and Senegal show that intervention scale-up reduces malaria burdens in the three age groups by similar proportions, but most cases and deaths are averted in under-5s. Proportional burden reductions are larger in lower-endemic settings, but numbers of cases and deaths averted for a given coverage increase are larger in higher-endemic settings, within and across countries. For DRC, given high ITN coverage but low effective case management coverage in 2015, case management is the intervention for which scale-up could have most additional impact by 2030 but programmatic inputs and resources to achieve this remain to be assessed by linking to the Spectrum costing module OneHealth Tool. In initial pilots, users appreciated the tool's alignment with monitoring indicators also as used in performance frameworks for program target setting and impact evaluation, and its graphical interface embedded in Spectrum's demographic platform for relatively quick comparison of policy scenarios.

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ASSESSING METHODS FOR ESTIMATING HOUSEHOLD BITING PROPENSITIES, SEASONALITY AND NOISE IN COUNTS OF MALARIA VECTORS

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Heterogeneity in malaria transmission varies with the intensity of transmission and it does not follow a Pareto rule. The distributions instead follow power laws across households or seasons in that the variance S and mean m are related by $S=am^b$. Understanding what drives these power laws and assessing the likely benefits of targeting requires having some quantitative understanding of heterogeneity and its underlying causes. These include seasonality, all the factors that make some households more attractive or easier to enter than others, environmental noise, and measurement error. The study focuses on mosquito counts data from an entomological surveillance conducted between October 2011 and March 2015 for 330 households at three study sites in Uganda and a simulation study investigating pseudo-data with known properties. We evaluate the performance of several statistical methods for partitioning the variance in mosquito biting into household biting propensities, seasonality, and environmental and measurement noise. We consider specific probability models that are capable of handling excess zeros while modeling non-zero counts properly. Seasonal adjustment of the time series data is performed using a range of temporal smoothing techniques, including a Gaussian smoothing kernel for sampling days and several Bayesian prior distributions for the temporally structured random effects. Using a range of model selection criteria, a zero-inflated negative binomial model was found to be robust and well-suited in modeling the mosquito counts across various simulated scenarios and real settings. The choice of the temporal smoothing technique differed slightly for each dataset. The information on household-level biting heterogeneity permits interventions to be targeted towards the locations of households with high malaria risk within them. Once transmission in an area has decreased but is maintained in hotspots of malaria transmission, such targeted interventions are likely to become increasingly important tools in malaria elimination efforts.

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SYSTEMS METABOLIC MODELING REVEALS DIFFERENTIAL NETWORKS PERTURBED AT PRIMARY INFECTION AND RELAPSE, IMPLICATING POTENTIAL BIOMARKERS FOR ACUTE AND CHRONIC MALARIA

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We applied Flux Balance Analysis (FBA) to model stage-specific metabolism of Non-Human Primate (NHP) model animals infected with *Plasmodium cynomolgi* or *P. coatneyi.* We derived genome-scale metabolic models (GEMs) for peripheral blood (PB) and bone marrow (BM), using transcriptomics data as constraints for metabolic fluxes. By bridging transcriptome and *in silico* metabolomics/fluxomics data, we identified key pathways that were perturbed during each stage of the parasite infection, highlighting key host metabolic modules in different tissues responding to parasite invasion. We further interrogated species-specific mechanisms by comparing GEMs constructed for *P. cynomolgi* and *P. coatneyi.* Focusing on purine metabolic pathway discovered by GEMs, we developed Generalized Mass Action (GMA) models to simulate kinetics of purine metabolism in PB and BM in *P. coatneyi* or *P. cynomolgi* infection. Notably, we found non-linear relationships between gene expression and corresponding fluxes. Tissue-specific comparisons showed that RNA metabolism is more active and more tightly regulated in BM compared to PB at primary infection, which is common for both parasites. Importantly, we observed positive correlation between increased fluxes toward hypoxanthine/uric acid and higher parasitemia in *P. coatneyi* infection, whereas in *P.cynomolgi* infection, the same fluxes were shown to be correlated with severity of disease. Strikingly, fluxes toward hypoxanthine and inosine were only shown to be constantly up-regulated across chronic infection in *P. coatneyi* but not in *P. cynomolgi* infection. Furthermore, this work derived a comprehensive map of temporal differential fluxes by longitudinal comparison in both *P. coatneyi* and *P. cynomolgi* infection, providing a resource for characterizing dynamics of purine metabolic pathways perturbed by malarial pathogens. These identified common and distinct fluxes and metabolites provide a paradigm to understand convergent and divergent molecular mechanism of host response to infections by two malaria species. This study showcases the application of metabolic modeling in biomarker discovery.

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JOINT MODELING OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX* INFECTIONS USING A BIVARIATE POISSON LOGNORMAL MODEL

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We utilized a bivariate Poisson lognormal model (BPLM) to estimate a covariate-adjusted association between *Plasmodium falciparum* and *P. vivax* infections and malaria clinical episodes. These two parasites are commonly seen as coinfections in Papua New Guinea (PNG). It is unclear whether they are positively associated with one another, or if one parasite suppresses the other. This was the primary motivation for fitting a BPLM, because it permits estimation of negative correlation, unlike most other multivariate Poisson models. We first simulated data similar to a cohort study that was conducted in PNG and compared two available methods for estimating the parameters of a BPLM. One method was a Bayesian model fit using MCMC methods and the other used adaptive Gaussian quadrature (AGQ) to estimate the model. We then estimated the association between two measures of burden from the PNG data using BPLMs: 1) the count of clinical episodes caused by *P. falciparum* and *P. vivax* parasites and 2) the count of genetically unique *P. falciparum* and *P. vivax* infections over an interval of observation. Our findings suggest that when the means of the two variables are large and exhibit overdispersion, it is possible to get estimates with low bias and correctly estimate the standard errors using either available method. However, when the means and variances are small, the MCMC method tends to produce biased estimates with slightly inflated standard errors and the AGQ method produces slightly biased estimates with extremely large standard errors. When the BPLM was fit to the PNG data, there was a moderate positive association between *P. falciparum* and *P. vivax* infections (correlation from BPLM 0.4, 95% CI [0.1, 0.8]), while adjusting for use of an insecticide treated bednet and age. Although there were higher rates of *P. vivax* infections (15.3 per child per year) in these children compared to *P. falciparum* (5.4 per child per year), there were more clinical episodes caused by *P. falciparum* (1.9 per child per year) than those caused by *P. vivax* (1.6 per child per year) over the entire cohort. We found no indication that *P. vivax* is protective against *P. falciparum* infections.

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PHARMACOKINETICS AND ACCUMULATION OF PIPERAQUINE WHEN USED FOR INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY (IPTP)

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While pharmacokinetic (PK) studies of dihydroartemisinin-piperaquine (DP) suggest that dose adjustment may not be necessary for treatment of malaria illness in pregnancy, similar data are needed for its use as intermittent preventive treatment in pregnancy (IPTp). We evaluated the PK and accumulation of piperaquine (PQ) when used as IPTp-DP in the context of a randomized controlled trial in Kenya. Among 371 HIV-negative pregnant women, 6, 57, 127, 121, and 60 received 1, 2, 3, 4, and 5 or more full 3-day treatment courses of IPTp, respectively. Plasma samples were collected at each antenatal care visit, following a breakthrough symptomatic malaria infection, and at delivery. PQ PK properties were evaluated using nonlinear mixed-effects modelling. PQ PK was described adequately by a 3-compartment disposition model with a flexible absorption model. Predicted median trough plasma concentrations accumulated (152%) during monthly IPTp; 10.7, 14.4, 15.7, 16.1, and 16.3 ng/mL following 1, 2, 3, 4, and 5 treatment courses, respectively. Simulations using the final PK model indicated much lower steady-state trough plasma concentrations of PQ after 5 treatment courses of IPTp at intervals of 45 (7.90 ng/mL) and 60 days (4.22 ng/mL) compared to monthly administration (16.3 ng/mL). Fifty pregnant women presented with breakthrough malaria infections at a median (IQR) observed PQ concentration of 4.94 ng/mL (2.80-8.65 ng/mL); no malaria occurred in women with a trough concentration above 34 ng/mL. No increases in adverse events were seen with increasing courses of IPTp-DP. Approximately monthly dosing of IPTp-DP resulted in PQ accumulation, though less than that reported among non-pregnant adults in Thailand (336%), likely due to the effect of pregnancy on PK properties. In the context of previously reported high efficacy, tolerability and safety, these data suggest that the standard DP dose regimen for *P. falciparum* is likely adequate and safe for monthly IPTp in a high malaria transmission area, with an advantage over bi-monthly IPTp. These are the first data on PK and accumulation of DP used for IPTp. Final PK modeling results will be presented.

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BROTHERS, SISTERS, AUNTS AND UNCLES: TRANSMISSION OF RELATED PARASITES IN POLYGENOMIC INFECTIONS OF *PLASMODIUM FALCIPARUM*

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Genomic analysis of natural *Plsmodium falciparum* populations can give us key insights into changes in population biology as malaria transmission is reduced following intensive interventions. Such data is valuable both as a direct assessment of transmission and in modeling the impact of interventions. There has been a strong correlation between declining genetic diversity and transmission intensity, suggesting that genetic diversity metrics could serve as proxies for declining transmission rates. Most studies have focused on the genetic diversity found within monogenomic infections, or those infections composed of a single parasite type. This is limiting and not representative of many transmission settings, since the proportion of multiple strain infections, or polygenomic infections, tends to be high. Polygenomic infections are largely assumed to be the result of superinfection, or infection by multiple mosquito bites, which would imply that the genetic diversity within polygenomic infections are directly linked to transmission intensity. However, the mosquito may influence the genetic diversity of parasites in subsequent infections, particularly if multiple strains are co-transmitted, or introduced simultaneously with a single mosquito bite. We analyzed the genetic relatedness of parasites within 32 polygenomic infections collected from Senegal and found that these infections are most likely the result of co-transmission. We also simulated the co-transmission process and found that the genetic diversity within polygenomic infections is influenced most strongly by the complexity of infection, or the number of unique parasite types, within the initial human host, and the number of oocysts that form within the mosquito midgut. Our results suggest that the genetic relatedness within polygenomic infections is a reflection of both the overall transmission intensity and the dynamics within the mosquito vector. We anticipate that polygenomic infections could greatly aid our understanding of transmission dynamics within malaria endemic regions, since it could be used to derive information from both the human and mosquito hosts.

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VECTOR "VACCINATION": OPTIMIZATION OF NON-MENDELIAN-BASED GENE DRIVES FOR MOSQUITO POPULATION REPLACEMENT

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As the efforts towards malaria control and elimination expand across socio-geographical strata, the penetration, accessibility, and correspondingly success rates of established interventions vary. Difficult to reach and/or dangerous locations render implementation of interventions requiring sustained complex logistical coordination and robust infrastructure hard, if at all feasible. Recently, advances in the CRISPR/Cas9-mediated constructs, along other genetic modification technologies, have allowed for gene drives altering mosquito populations at scale. Homing endonucleases, TALENs and more recently CRISPR constructs, for instance, could be used to express anti-falciparum antibodies in target mosquito species. We implemented such a gene drive, replacing wildtype populations with mosquitoes resistant to Pl. falciparum within the EMOD modeling framework. We studied four distinct use cases in Tanzania, Nigeria, DRC, and Somalia - each characterized by distinct climates, disease prevalence and mosquito densities, etc. -- representing a realistic range of mosquito habitats and seasonality intensity. We investigated the optimal parametrization of genetic constructs - e. g. in terms of fitness penalty tolerance, infection modification and population replacement rates - along with gene drive logistics - e.g. mosquito release numbers, schedules, etc. We demonstrate feasible parameter ranges, where infection modification gene drives can replace wildtype mosquito populations in each of the four use casses. We caution that the resulting parasite prevalence and hence the success of a gene drive depends significantly on the infection modification rate and provide the respective thresholds for successful genetic constructs. The robustness of parasite prevalence reduction under different gene drive strategies in all use cases was evaluated with respect to malaria re-importation and mosquito species heterogeneity.

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PENTOSE PHOSPHATE PATHWAY INHIBITION ELEVATES OXIDATIVE STRESS AND IMPEDES FECUNDITY IN *ANOPHELES GAMBIAE*

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Blood digestion in hematophagous insects is associated with elevated reactive oxygen species (ROS) resulting from pro-oxidant heme ingestion. Mosquito capacity for defense against oxidative stress is limited and an overabundance of ROS can lead to reduced fecundity and death. Reducing power from internal sources is essential for oxidative stress defense which can impact insecticide sensitivity and defense against pathogens. The primary system used to alleviate oxidative stress is the pentose phosphate pathway (PPP). The major role of PPP is the regeneration of NADPH by reducing NADP+, and 6AN is a competitive inhibitor of G6DPH, the rate-limiting enzyme of PPP. PQ is an exogenous stress inducer, and PPP inhibition results in the accumulation of endogenous stress. We examine the dynamics of oxidative stress by induction by paraquat (PQ), inhibition of the PPP by 6-aminonicotinamide (6AN) and alleviation of oxidative stress by lycopene by oral feeding followed by egg counts and biochemical assessment of females. We hypothesis that PQ and 6AN will induce oxidative stress and result in reduced fecundity. Lycopene should rescue this phenotype by scavenging ROS. Both PQ and 6AN feeding increased oxidative stress levels and decreased fecundity. Co-feeding with lycopene attenuated these adverse effects. 6AN when fed with PQ results in a normal egg number possibly due to inactivation of NADPH production which is required for PQ toxicity. Lycopene also improves 6AN reduction in egg number suggesting that lycopene can alleviate ROS species induced by reducing NADPH production. 6AN reduced NADPH production resulting in a high NADP+:NADPH ratios indicating that the PPP is inhibited by 6AN in our model system. GSSG:GSH ratio also was increased by both 6AN feeding and PQ indicating both of these compounds result in increased oxidative stress. These antioxidants and pro-oxidants can provide a manipulatable link between mosquitoes and egg production capacity. This knowledge can be used to design novel and effective vector control strategies which may influence insecticide sensitivity, infection susceptibility, fecundity and longevity.

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INVESTIGATING *ANOPHELES FUNESTUS* SUSCEPTIBILITY AND IMMUNE RESPONSE TO *PLASMODIUM FALCIPARUM* INFECTION

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Anopheles funestus is a major vector of malaria in Africa. However, because it is difficult to colonize, research on this mosquito species has lagged behind other vectors, particularly the understanding of its susceptibility and interactions with the *Plasmodium* parasite. In order to fill this important knowledge gap, experimental infections were conducted from March to June 2015. 3-5 day old *An. funestus* F_1 mosquitoes derived from wild-caught females from Cameroon were fed with infected blood taken from gametocyte carriers using an artificial glass-parafilm feeding system. Feeding rate was recorded, and fed mosquitoes were dissected at day 7 for oocysts count. Comparative and parallel experiments were performed with the known *Plasmodium*-susceptible, *An. coluzzii* Ngousso laboratory strain. Microarrays analysis was performed to assess the molecular basis of *An. funestus* immune response to *P. falciparum* invasion.The results revealed that *An. funestus* displays a similar level of susceptibility to *Plasmodium* infection compared to *An. coluzzii*. The prevalence of infection in fed *An. funestus* mosquitoes was 38.52% (range: 6.25-100%) and the median oocyst number was 12.5 (range: 1-139). In parallel, the prevalence in *An. coluzzii* was 39.92% (range: 6.85-97.5%), while the median oocyst number was 32.1 (range: 1-351). Genome-wide microarray-based transcription analysis showed that *An. funestus* innate immune system is activated during midgut invasion. A total of 222 genes were found to be differentially expressed between infected and non-infected mosquitoes including several known immune response genes such as C-type Lectine, APL1 family genes, LRIM1, Serine protease, Serine collagenase and niemann-pick type c. However, genes such as TEP1 were not over-expressed in contrast to *An. gambiae* suggesting possible differences between both species. The high susceptibility of *An. funestus* to *P. falciparum* and its widespread distribution across Africa highlight the need to also tackle this vector for significant malaria vector control program.

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ELUCIDATING THE ROLE OF LIPOGENIC AND LIPOLYTIC PATHWAYS IN MOSQUITO REPRODUCTION AND *PLASMODIUM FALCIPARUM* TRANSMISSION

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Female *Anopheles* mosquitoes undergo a number of blood feeding cycles on a vertebrate host in order to produce multiple egg batches, and these obligatory steps in the mosquito life cycle are exploited by *Plasmodium* parasites for their own transmission. Blood feeding is therefore a key step for both mosquito reproduction and parasite transmission. Indeed these two processes are temporally and physiologically coupled and can be exploited to impact malaria dynamics in endemic areas. Previous studies revealed a correlation between blood meal digestion and major changes in transcriptional profiles of metabolic genes involved in lipid biosynthesis, transport, and breakdown, suggesting the occurrence of de novo lipid synthesis triggered by blood feeding followed by lipid mobilization. Here, we aim to elucidate the specific role of blood meal-derived lipids (and/or of lipids synthesized de novo after a blood meal) in *Anopheles* reproduction and parasite development in mosquito stages. To address this, we performed targeted depletion of key lipogenic and lipolytic enzymes in the main African malaria vector using RNA interference (RNAi) and assessed their impact on oogenesis and *P. falciparum* infection. Strikingly, knockdown of acetyl-CoA carboxylase (ACC), one of the rate-limiting enzymes in de novo fatty acid synthesis (lipogenesis), reduced egg development and *P. falciparum* infection in *An. gambiae*. On the other hand, inhibition of triglyceride (TAG)-lipase, involved in lipolytic breakdown of TAGs to yield free fatty acids and diacylglycerol (DAG), had opposing effects on egg development and *Plasmodium* infection: depletion of TAG-lipase significantly impaired the number of eggs developed but resulted in a significant increase in oocyst size without any apparent impact on the number of oocysts per midgut. The latter results suggest occurrence of possible scavenging of host TAGs by malaria parasites to meet developmental needs within the *Anopheles* vector. While further characterization is underway, these data provide the first direct evidence of the requirement of host lipids by human malaria parasites for successful transmission.

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DISRUPTING STEROID HORMONE SIGNALLING IN ADULT *ANOPHELES GAMBIAE* FEMALES BLOCKS *PLASMODIUM* DEVELOPMENT AND OFFERS ALTERNATIVE TARGETS FOR MOSQUITO CONTROL

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Human malaria is a major public health burden in tropical and subtropical countries and is transmitted exclusively by female *Anopheles* spp. mosquitoes. Malaria control strategies based upon the deployment of pyrethroid-impregnated long lasting insecticide treated nets (LLINs) are threatened by the continued spread and intensification of insecticide resistance. This represents a major obstacle to malaria elimination and as such new means of protection against the mosquito vector are desperately needed. Here we report that treatment with non-steroidal ecdysone receptor agonist dibenzoylhydrazines (DBHs) such as methoxyfenozide impact a number of parameters key to the vectorial capacity of the principal malaria vector *Anopheles gambiae*. Topical application of methoxyfenozide causes extensive apoptosis in the primary ovarian follicles of treated females, significantly reducing egg production while also impacting lifespan, oviposition and - in virgin females - mating refractoriness. Importantly, application of methoxyfenozide prior to females receiving an infectious *Plasmodium falciparum* blood meal triggered up to an 87% reduction in oocyst prevalence 7 days after blood feeding, suggesting that female susceptibility to infection is mediated in part via ecdysone signalling. This work provides critical insights into vector/parasite interactions and - in the face of the potential collapse of existing vector control methods - sets forth the case for the use of DBH compounds in malaria control strategies.

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THERE AND BACK AGAIN: A MOSQUITO SPERM'S JOURNEY FROM INSEMINATION TO FERTILIZATION

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Interfering with mosquito reproduction to control vector populations holds significant promise. Most investigative effort to develop novel control targets has focused on females, yet male contributions to reproduction are often overlooked. In particular, sperm biology and sperm's movement through the female reproductive tract are poorly understood, but studying sperm may provide various opportunities to interrupt reproduction. During insemination, sperm are deposited in a semen-receiving organ, where they display hyperactivated motility. They quickly localize to ducts leading to long-term storage organs called spermathecae. Within minutes, they travel up these ducts and are maintained by the female for her entire life. Ultimately, they are carefully released for fertilization as eggs are laid. Completion of this journey requires the precise coordination of motility, interactions with the ejaculate, nourishment by the female, and possibly modifications to sperm that make them fertilization competent. These

processes have been superficially examined microscopically, but very little is known about how sperm function on the molecular level. We discuss what is known of mosquito sperm's path through the female, highlighting areas for future exploration and discussing possible molecular targets that could be exploited by vector control strategies. We also provide unprecedented footage of mosquito sperm motility inside the female reproductive tract that will aid our understanding of how sperm function *in vivo*.

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SICPIN, A MULTIFUNCTIONAL IMMUNOMODULATORY SALIVARY PROTEIN FROM THE BLACK FLY *SIMULIUM NIGRIMANUM*

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Hematophagy is key to blood feeding arthropods reproductive success and an important link in pathogen transmission cycles. Salivary gland homogenates from blackflies have been shown to contain immunomodulatory activity on murine splenocytes. However, the molecule(s) responsible for this salivary activity remains elusive thus far. Here, we report the first immunosuppressive protein from blackfly salivary glands. Sicpin (*Simulium* cell proliferation inhibitor) was produced in *E. coli* and purified using size exclusion and ion exchange chromatography. Sicpin inhibited cell proliferation in a dose-response manner independently of the mitogen utilized (ConA, LPS, CD3/CD28 and Pokeweed). LPS or ConA stimulated cells had a significant lower proliferation rates ($P<0.001$) in the presence of Sicpin (IC50=0.5μM) with 10uM completely abrogating cell proliferation. Flow cytometry analysis showed that Sicpin inhibits proliferation of CD19+ B-cells and CD4+/CD8+ T-cells; also inhibiting antigen-specific cell proliferation without inducing apoptosis in resting or mitogen-induced splenocytes. The production IFN-α, IL4, IL5, IL6 and IL10 by splenocytes stimulated by ConA or LPS was dose-dependently reduced by Sicpin. Additionally, carrageenan-induced paw-edema model showed that the intensity of edema significantly decreases in the presence of Sicpin. The molecular mechanism of Sicpin on cell proliferation inhibition is currently under investigation; however, initial binding experiments using SPR analysis showed a direct binding to soluble CD4 receptor with a calculated KD of 17.77 nM. Direct binding of Sicpin to CD4 could inhibit the subsequent TCR ligation-induced T cell signaling at the earliest steps including tyrosine phosphorylation of the receptors, downstream effector proteins, and lipid raft reorganization. The immune suppressive and anti-inflammatory properties of Sicpin should be explored as a strategy to modulate immune responses in infection and tumor proliferation as well as its involvement in parasite transmission.

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A LOOK AT TWO FACTORS THAT MODULATE *AEDES AEGYPTI* MOSQUITO VECTOR COMPETENCE FOR DENGUE VIRUS

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Dengue virus (DENV) is a single stranded, RNA virus principally transmitted by the *Aedes aegypti* mosquito. While there is currently no vaccine or treatment available for those infected with the virus, DENV transmission can be prevented via vector control strategies. However, both biological and physical methods that have been employed thus far have had minimal success in curtailing the virus; and without a thorough understanding of how the mosquito modulates DENV infection; we may never be able to achieve this goal. Studies have shown that interactions between the mosquito's immune system, the pathogen, and the mosquito's endogenous midgut microbiota are critical determinants of the outcomes of transmission. Viral replication in the midgut results in the activation of a signaling cascade that turns on the mosquito's immune response. Thus far, we have isolated two key restriction factors, DVRF1 and DVRF2, which are downstream effectors of the JAK-STAT immune signaling pathway that have anti-dengue activity. But very little is known about how these specific effector molecules mediate this anti-pathogenic effect. The natural microbiota of mosquitoes is also a great influence on the mosquito's biology. Interactions between the mosquito microbiota and the virus also modulate vector competence for DENV infection via mechanisms such as immune response activation, anti-viral molecule production and resource competition. We have started the process of screening several mosquito-gut associated fungus for their effects on DENV susceptibility, from which we hope to characterize their mechanisms for infection modulation. Studying theses two important factors that modulate DENV infection is a step in the right direction toward developing alternative strategies for viral transmission control.

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CHIKUNGUNYA FEVER IN CLINICALLY DIAGNOSED PATIENTS: COMPARATIVE STUDY BETWEEN LABORATORY CONFIRMED VERSUS NEGATIVE CASES DURING THE 2015 OUTBREAK IN YUCATAN, MEXICO

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In the second half of 2015, chikungunya and dengue outbreaks took place simultaneously in the state of Yucatan, Mexico. This coexistence of both outbreaks posed a challenge to differential clinical diagnosis. During the outbreak, only a subsample of chikungunya dengue cases were confirmed by laboratory standard methods. This study was undertaken to identify signs and symptoms useful for clinically discriminate chikungunya from other endemic infections in early febrile stage. In this comparative, cross sectional study, we analyzed the data from the chikungunya surveillance cases at the main public general hospital in the state of Yucatan, including for our analyses only those whose blood samples were referred for its confirmation by the epidemiologic reference laboratory in the period between August and December of 2015. We compared the clinical manifestations of confirmed cases versus discarded cases using a logistic regression model. We included 181 of which 152 tested positive for Chikungunya virus, finding that pruritus is a suggestive symptom of an acute infection caused by CHIKV. Osteoarticular manifestations did not differ significantly, between confirmed or discarded cases, but pruritus was twice as common among chikungunya confirmed cases. Pruritus was a suggestive symptom of an acute infection caused by CHIKV. In 2015, Yucatan Mexico experienced a simultaneous occurrence of chikungunya and dengue outbreak. Clinical differentiation between CHIKV and dengue represented a diagnostic challenge, after statistical analyses, we can conclude that pruritus was an early suggestive symptom of an acute infection caused by chikungunya.

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EVOLUTIONARY INFLUENCES ON THE REDUCTION IN ENZOOTIC CIRCULATION AND HUMAN INCIDENCE OF WESTERN EQUINE ENCEPHALITIS

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Understanding the evolutionary and ecological circumstances in which arboviruses emerge into naïve geographical areas is critical for the development of targeted maintenance and prevention strategies. This

need was highlighted by the recent emergence of chikungunya and Zika viruses in the Americas. In order to develop a complete understanding of the ways in which viruses emerge, the factors surrounding a reduction in virus activity (submergence) must also be studied, and Western equine encephalitis virus (WEEV) provides a unique opportunity to study this. WEEV caused several epizootic events in the early 20th century that account for the death of thousands humans and equids. However, the last human case in North America occurred in 1994 even though virus can still be detected in mosquito pools, albeit at reduced levels. Previously, we identified six nonsynonymous mutations that were phylogenetically significant and have a phenotypic effect on WEEV's enzootic hosts. Competitive fitness assays show contemporary mutations have a competitive advantage in both *Culex* tarsalis and house sparrows, but have no effect on virulence in the Syrian golden hamster. These data suggest mutations have accumulated by positive selection only enhance the ability to transmit between its enzootic hosts and vector. We also hypothesize the mutations that confer mammalian virulence were purified out of the population by negative selection due to a reduction in selective pressure on those residues. Overall, the evolutionary profile of WEEV over the 20th century trends away from disease in mammals and toward its enzootic cycle. Several factors could account for this including vaccination and drastic reduction of the US equine population, the use of screens on windows and doors, and/or changes in irrigation practices. In summary, the submergence of WEEV presents a case study where its reduction was likely precipitated by an ecological shift critical for the virus' maintenance of the mutations that connote virulence in mammals and subsequently compensated by increasing its adaptability to its enzootic hosts.

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THE BURDEN OF CHRONIC CHIKUNGUNYA DISEASE AND QUALITY OF LIFE IN CURAÇAO

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The burden of chronic chikungunya disease and quality of life in Curaçao Curaçao, an island in the southern Caribbean Sea, was affected by a major chikungunya (CHIK) epidemic in 2014 resulting in an estimated 25.000-30.000 cases of CHIK at the end of the outbreak (January 2015). After the epidemic culminated, CHIK remained a public health problem given the chronic phase of the disease which can include joint pain, arthritis, fatigue and depression for up to five years. However, studies on the chronic phase of CHIK remain scarce, especially in the Americas. The aim of this study was to estimate the burden of the CHIK outbreak in Curaçao in terms of duration of disease, symptoms and impact on quality of life 3-16 months after diagnosis. Following the CHIK epidemic of 2014-2015 on Curaçao, a comprehensive cross-sectional survey was performed in June and July 2015. A total of 411 adult participants were contacted and invited to join the study, of which 339 consented (response rate= 82.5%). Interviews took place 92-460 days after disease onset. Of those interviewed, 306 individuals had a laboratory confirmed CHIK virus infection. Symptoms of the chronic disease course were evaluated, and quality of life was assessed using the RAND-36 score. We will present a comparison of the fully recovered and the still affected population of chikungunya patients. The mean age of the participants was 52 years (range: 18-94 years). Preliminary results show that the degree in which subjects were affected by chronic chikungunya disease differs: 37.2% were defined as fully recovered, while the remaining 62.8% were defined as still being mildly affected (35.7%) or highly affected (27.1%). Of the total population, only 21.4% reported to be fully recovered from CHIK within one month. We will give a detailed description of the clinical spectrum of chronic chikungunya disease and will show the impact on the quality of life aspects reported by the studied population. We believe that this comprehensive study will provide important insight in the disease course of chronic chikungunya in the Americas and its impact on its population.

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CHIKUNGUNYA EPIDEMIC IN CARABOBO STATE, VENEZUELA 2014: A STUDY ON EPIDEMIOLOGICAL DEVELOPMENT, CLINICAL MANIFESTATIONS AND RISK FACTORS

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Chikungunya (CHIK) was a relatively uncommon and poorly documented illness. However, in the last decade this re-emerging viral vector-borne disease caused an increasing number of outbreaks in the tropical and subtropical regions of Africa and Asia. At the end of 2013, CHIK reached the Americas spreading rapidly through most countries. By mid 2014, Venezuela was hit by a devastating CHIK epidemic that swept the country with an estimated attack rate of 60%. Carabobo State, one of the first Venezuelan regions to be affected, reported its first autochthonous case in June 2014. We aimed to characterize the epidemic, clinical manifestations and risk factors associated with CHIK transmission in Carabobo State. Epidemiological and clinical data of patients attending health centers was obtained from the surveillance system of the Regional Ministry of Health. Between June-December 2014, data from 613 patients were included, of which 167 laboratory confirmed (103 (61.7%) positive). Univariate and multivariate analysis of laboratory-confirmed and suspected cases were performed. We detected an epidemic peak in week 34, 74 days (10.6 weeks) after the first reported case. The mean of the epidemic curve was 14.3±3.7 weeks. A two week lag was observed between the time of symptom onset and that of case notification. In laboratory confirmed patients, rash alone (OR=3.39, p=0.018) and the combination of fever, rash and arthralgia (OR=2.39, p=0.066) were associated with CHIK. Housewives and domestic workers had 79% lower risk of being CHIK positive (p=0.050). Similar findings were obtained when comparing the study group of suspected and laboratory-confirmed CHIK cases showing a relatively accurate clinical diagnosis from physicians. In addition, crowding (≥1.5 people/bedroom), a marker for lower socio-economic status, was positively associated with acquiring CHIK (OR=1.75, p=0.059). Our findings may add to the current diagnosis guidelines and give insights about CHIK associated risk factors in Venezuela. Additionally, by improving the time response of case notification, earlier preventive measures may be put in place to reduce CHIK transmission.

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SEASONAL PREVALENCE OF ALPHAVIRUSES AND FLAVIVIRUSES IN CHILDREN IN WESTERN KENYA

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Arboviruses present a significant threat in many regions of the world, continuing to spread through explosive expansion to previously unaffected areas. Many elements may influence the dynamics of exposure through environmental, behavioral, and biological factors, as well as the related spectrum of disease. This study aims to describe the prevalence of alphaviruses, such as chikungunya virus (CHIKV) and o'nyong n'yong

virus (ONNV), and flaviviruses, such as dengue virus (DENV) and West Nile virus (WNV), in western Kenya over time. Serum samples were taken from healthy afebrile children in two inland communities, Chulaimbo and Kisumu, at enrollment and during a 3-month follow up visit. Enrollment took place in March-May of 2014 for Kisumu, and October-December 2014 for Chulaimbo. Questionnaires on health history, socioeconomic status, home environment, and mosquito exposure were collected during each visit to determine risk for arbovirus transmission. Sera were tested using indirect IgG ELISAs using CHIKV and DENV antigens to identify previous exposure to alphaviruses or flaviviruses (cross-reactivity within each viral genus is common). Of 748 children, 8% (CI95 6-10%) were seropositive for CHIKV indicating previous exposure to alphavirus, and 11% (CI95 9-13%) were seropositive for DENV indicating previous exposure to a flavivirus. At follow-up, 30% (CI95 25-36%) and 36% (CI95 32-39%) of participants were seropositive for alphaviruses and flaviviruses, respectively. Flaviviruses seropositivity was significantly higher in Chulaimbo ($p = 0.01$) at follow-up in January-March 2015. Comparatively, prevalence at the time of the follow-up visit differed significantly between villages for both alphaviruses ($p = 0.0004$) and flaviviruses ($p < 0.0001$). These data indicate suggest an event, such as flooding with an increase in local mosquito populations increased exposure. Because alphavirus infections increased prior to June and flavivirus infections prior to January, their exposure may also correlate with seasonal variation. Additional ecological, social, and demographic risk factors will be discussed.

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RISK FACTORS FOR CHIKUNGUNYA PATIENT HOSPITALIZATION — PUERTO RICO, 2014

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The Sentinel Enhanced Dengue Surveillance System (SEDSS) is an acute febrile illness (AFI) surveillance and research platform that determines the etiology and clinical outcome in febrile patients presenting to two hospitals in Puerto Rico. Chikungunya emerged in the Caribbean in late 2013, and diagnostic testing was incorporated into SEDSS in March 2014. The objectives were to estimate the proportion of chikungunya virus (CHIKV) infection in patients presenting with AFI, estimate the incidence and identify risk factors for chikungunya patient hospitalization. Demographic and clinical information were collected at presentation. Medical records were reviewed to collect information about clinical manifestations and outcomes. Of 3,035 patients enrolled in SEDSS during May-December 2014, 1,469 (48%) had confirmed CHIKV infection by RT-PCR. In total, 157 (10.7%) patients with evidence of CHIKV infection were hospitalized, 6 (0.4%) were admitted to the intensive care unit, and 2 (0.1%) died. Median age among hospitalized and non-hospitalized patients was 10 years (range: 0 to 93) and 26 years (range: 0 to 97), respectively (p-value=0.00). Rate of hospitalization was highest in infants (67%) and the elderly (17%). Neither the presence of co-morbid conditions nor day of presentation for care post-illness onset were associated with patient hospitalization. Clinical and laboratory findings associated with hospitalization (relative risk >1.5 or p-value= 0.00) included white blood cells, hematocrit, platelet count, pale or cold skin, skin rash, bruises, cyanosis, seizures, and irritability. Additional analyses will adjust for age in identification of risk factors for chikungunya patient hospitalization. The most common atypical manifestations among hospitalized patients were cardiac arrhythmia (7%), encephalitis (4%), and vesiculobullous skin lesions (3%). Fatal cases were associated with exacerbation of underlying chronic medical conditions. Although chikungunya is a self limiting illness, some patients - particularly infants and the elderly - may develop severe manifestations and merit closer monitoring.

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PROTEIN SPECIFICITY OF ANTIBODY RESPONSES TO SOUTH AMERICAN ALPHAVIRUS INFECTIONS USING A NOVEL MULTIPLEXED ASSAY

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Arboviruses are a leading cause of emerging and re-emerging diseases around the world. Members of the Alphavirus, Flavivirus, and Bunyavirus genera are primarily responsible for causing disease in humans, with infections ranging from asymptomatic to mild symptomatic, and can sometimes turn severe. Because many arboviral infections cause mild, undifferentiated symptoms, illnesses are often undiagnosed or misdiagnosed, especially in Dengue-endemic regions, often leading to improper estimations of disease incidence rates. In addition, diagnostic testing may be attempted post-acute phase after viral clearance, often limiting detection of past infection to serological-based assays only. Unfortunately, commercially available serological diagnostics are not readily available for many such diseases, and antibody cross-reactivity with related, non-etiologic agents may occur, leading to misdiagnosis. To gain a better understanding of disease prevalence, particularly in resource-limited areas, a detection platform with high specificity is needed to cover a wide range of endemic and emerging diseases. Towards this goal, we have developed a multiplexed alphavirus protein microarray to perform serological-based assays to identify specific viral exposures. The protein microarray contains three purified, recombinant structural proteins from multiple arthrogenic or encephalitic alphaviruses, including Chikungunya virus (CHIKV), Mayaro virus (MAYV), and Venezuelan Equine Encephalitis virus (VEEV). To detect specific antibody binding to alphaviral antigens, convalescent sera from patients in South America with PCR-confirmed alphavirus exposures were tested for antibody binding to antigens in the protein microarrays. In particular, IgG in early convalescent sera from patients with MAYV, VEEV or CHIKV infections were tested for specific antigen binding. We determined that by using purified alphavirus proteins, we were able to detect IgG binding to one or more specific viral antigens, with minimal cross-reactivity occurring with viral antigens with high levels of sequence homology.

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ALTERING BLOOD BRAIN BARRIER PERMEABILITY: HOW ROUTE OF INFECTION, CYTOKINE INDUCTION AND HEPARAN SULFATE BINDING CONTRIBUTE DURING ENCEPHALITIC ALPHAVIRUS INFECTIONS

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Alphaviruses are arthropod-borne, enveloped viruses that contain a single-stranded, positive-sense RNA genome. Alphaviruses are generally categorized into two categories based on geographic location and disease manifestations: old world alphaviruses mainly cause febrile/arthritogenic disease while new world alphaviruses can cause encephalitis. Encephalitic

alphaviruses have a range of morbidity and mortality in humans with the most virulent causing ~40% mortality, while all can cause permanent neurological sequelae. There are currently no licensed vaccines or antiviral therapies, therefore it is important to understand how the encephalitic viruses enter the central nervous system (CNS) and alter the blood brain barrier (BBB) so that therapeutic strategies can be designed to target these events. Our initial hypothesis, based on previous studies, was that an initial early opening of the BBB occurs, presumably due to cytokine induction, allowing the virus to gain entry into the CNS, and then a later opening of BBB occurs due to viral replication within the CNS. Our goal is to determine if route of exposure (subcutaneous vs aerosol) and the ability to bind heparan sulfate (HS) alters entry into the CNS and permeability of the BBB. Using fluorescently labeled molecules of different molecular weights, our data suggest that there is an initial opening of the BBB for lower molecular weight molecules, likely due to cytokine induction, but it may not be large enough for the alphaviruses to enter into the CNS. Additionally, the opening of the BBB to larger particles, of similar size to a virion, does not occur until late in infection. Interestingly, route of infection can play a role in the ability of certain encephalitic alphavirus to cause leakage of the BBB to small molecules, but not for all the encephalitic alphaviruses and this may be greatly influenced by the ability to naturally bind HS. Taken together, our data suggest that encephalitic alphaviruses do not alter the permeability of the BBB to gain entry into the CNS, however route of infection, innate immune responses and bind HS binding impact the ability each virus to induce BBB permeability.

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COMPREHENSIVE MUTAGENESIS OF DENGUE VIRUS ENVELOPE PROTEINS TO MAP ANTIBODY EPITOPES AND IDENTIFY RESIDES ESSENTIAL FOR FUNCTION

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To characterize the immune response to dengue virus (DENV) infection, we have developed a high-throughput strategy that enables the rapid identification of both linear and conformational epitopes on DENV prM/E envelope proteins from all four DENV serotypes. For each DENV serotype (1-4), we used Shotgun Mutagenesis technology to create a comprehensive library of single mutations in DENV prM/E, 3,380 mutations in total. Each library of individual mutant expression plasmids was arrayed into 384 well plates and transfected into human cells to achieve native protein expression and folding. The immunoreactivity of MAbs to the prM/E variant in each individual well was quantified by high-throughput flow cytometry, resulting in approximately 200 MAb epitopes. The epitopes obtained have been correlated with their abilities to protect against DENV infection. We have also produced DENV virions from all four mutation libraries using a previously developed DENV reporter virus particle (RVP) system, allowing us to screen each individual DENV Env variant protein for DENV particle budding and infectivity. For DENV3, analyses of budding and infectivity, along with the MAb binding studies, identified residues whose mutation eliminated virus infectivity, but did not impact E protein expression, antigenicity, virion assembly, or particle budding. We identified variants that showed increased DENV virion budding, up to 5-fold above wild-type, indicating the ability to engineer highly expressed, non-infectious DENV variants that can be used for vaccine design. Our research has identified neutralizing epitopes in DENV prM/E and specific sites that are critical for DENV infectivity, providing new targets and opportunities for vaccine development.

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THE CLINICAL OVERLAP OF SEVERE DENGUE CATEGORIES

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The WHO (2009) classification has the following three categories of severe dengue i) dengue shock / respiratory distress with fluid accumulation (vascular leakage), ii) severe bleeding, and iii) severe organ manifestation. The prospective multicentre DENCO study provides one of the largest datasets of hospitalised dengue patients from 7 Countries in Asia and Latin America (N=1734). All patients were followed daily throughout the evolution of their illness by trained physicians using a single comprehensive case report form. Here we analyse the frequency and overlap between these categories in 271 confirmed severe dengue patients. The overall proportion and categories of severe disease did not differ significantly between Asia and Latin America, but between age groups. Severe dengue occurred in 17.3% of children (< 15 years) compared to 12.9% of adults (≥ 15 years). Within the severe children, 94.0% were diagnosed with vascular leakage and 12.0% with severe bleeding, whereas in severe adults 81.6% had vascular leakage and 19.5% severe bleeding. The trajectories of the platelet counts between patients with severe bleeding and severe vascular leakage diverged at day of illness 3 with significantly lower platelet counts in patients with severe bleeding on days 5 and 6. Severe vascular leakage occurred in 244 patients (90% of all severe patients), of whom 213 (87%) did not experience severe bleeding or severe organ dysfunction, 210 were diagnosed with clinical shock, 79 with fluid accumulation with respiratory distress, and 45 with both clinical shock and fluid accumulation with respiratory distress over the course of the illness. Severe bleeding and severe organ dysfunction accounted for 39 and 28 cases respectively, of whom 12 (31%; severe bleeding) and 14 (50%; severe organ dysfunction) did not overlap with the other categories. Thus, the majority of the vascular leakage cases and 30-50% of the cases with severe bleeding or severe organ manifestations were classified as severe in only one category, highlighting the usefulness of these categories and their potential as research endpoints.

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A PHASE 1 EVALUATION OF THE SAFETY AND IMMUNOGENICITY OF RDEN3Δ30 AS A DENGUE 3 HUMAN CHALLENGE STRAIN

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As human experimental medicine models become important in the testing of candidate dengue vaccines, we developed a Dengue 3 model. DENV-3 Sleman/78, isolated from central Java, and was associated with a clinical illness milder than from other circulating DEN3 strains. To attempt to further attenuate the strain for possible use as a candidate DENV-3 vaccine, 30 nucleotides were removed from the 3′ untranslated region and the virus was designated rDEN3Δ30. During pre-clinical testing of this virus in rhesus macaques, rDEN3Δ30 was indistinguishable from the DEN3 Slemen/78 wild type parent virus; there was no difference in mean peak titer of virus or number of days of viremia. For this reason, rDEN3Δ30 was evaluated as a potential DENV-3 challenge virus in a dengue human infection model. We describe the clinical manifestations and viremia associated with this DENV strain in healthy human subjects, and its future role as part of DENV challenge studies.14 subjects were enrolled;10 subjects received 103 PFU of DENV3 vaccine and 4 received

placebo. Viremia and safety labs were measured at Days 2,4,6,8,10,12 and 16. Subjects were followed for evidence of illness or fever alternating days (QOD) in clinic or by phone. The most common side effects headache and fatigue were the same in rDEN3Δ30 recipients and placebos. 80% of rDEN3Δ30 recipients developed a transient Dengue-like rash and 100% had rDEN3Δ30 recovered from the blood; no subject had fever. The major lab findings in vaccines were mild thrombocytopenia (n=2) and one mild Neutropenia. No SAEs were reported. This was first time rDEN3Δ30 was administered to healthy human volunteers. No volunteer developed fever or dengue like illness. Overall, the strain appeared well-tolerated with the exception of mild- moderate transient rash in 80% of vaccines. The virus was recovered from 100% of treated subjects. The high incidence of viremia and rash induced by this virus supports its use in dengue human infections studies. Previous studies have demonstrated the LATV formulation prevents against infection with DEN2 challenge. The results of this phase 1 study will provide a foundation for future challenge studies.

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ANALYSIS OF THE CURRENT MAJOR DENGUE OUTBREAK IN ARGENTINA IN AN AREA WITH PERMANENT CONTROL ACTIVITIES AGAINST *AEDES AEGYPTI* SINCE 2009

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The city of Tartagal (Salta Province) is located in the northeast of Argentina, 100 km north of the Tropic of Capricorn and 50 km south of Bolivia. It has a population of 69,225 inhabitants and 18,052 households. Mundo Sano has a Surveillance and Vector Control Program for *Aedes aegypti* in place since October 2009 in collaboration with the Municipality of Tartagal with the objective of diminishing the incidence of dengue cases in the locality. Until epidemiological week (Epi week) 14 of 2016, the quantity of dengue infected people registered in Argentina reached 55,431 (2,909 from Salta), with circulation of both DEN1 and DEN4 serotypes. In Tartagal, the number of infected people until the same Epi Week was 106. The last major epidemic in Argentina occurred in during 2008/2009, where Tartagal presented 665 cases, including the first death by severe dengue reported in in the country. The actions included in the program for integrated control of *Aedes aegypti* and the eco-epidemiology of dengue in Tartagal are the following: a) Focal Cycles (during the Summer Season), b) Entomological monitoring (throughout the entire year), c) evaluation of the gonadotrophic activity through the use of ovitraps (throughout the entire year), d) environmental manipulation to remove breeding sites (previous to the Summer Season), e) organization and development of focal blocking activities (in coordination with the J.D. Perón Hospital from the city of Tartagal), e) capture of adults for the detection of viral infection and f) evaluation of insecticide resistance in *Ae. aegypti* populations in the city. The objective of the present study is to show the importance of a permanent Surveillance and Vector Control Program, with planned activities, continued and uninterrupted, in order to reduce the abundance of *Ae. aegypti* through the control of larval stages and their breeding sites, especially during outbreaks of dengue and other arboviruses transmitted by *Ae. aegypti*.

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DISSECTING ANTIBODY RESPONSE INDUCED BY CHIMERIC YELLOW FEVER-DENGUE, LIVE-ATTENUATED, TETRAVALENT DENGUE VACCINE (CYD-TDV) TO UNDERSTAND VACCINE EFFICACY IN NAÏVE AND DENGUE EXPOSED INDIVIDUALS

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Dengue vaccine development is complicated by the presence of 4 virus serotypes. Sanofi Pasteur has developed a chimeric yellow fever-dengue, live-attenuated, tetravalent dengue vaccine (CYD-TDV) that is approved for use in children >9 years of age in several countries. In two large scale phase III efficacy trials, CYD-TDV was efficacious at reducing laboratory-confirmed dengue cases. Efficacy varied by serotype, in addition to being higher in DENV exposed than in DENV-naïve participants. While these results are encouraging, they highlight the complexity of human immune response to vaccination, which is often not balanced across the 4 serotypes and is influenced by prior immune status of vaccinees. Our study compare the properties of DENV-specific antibodies in naïve and DENV exposed individuals who received three doses of CYD-TDV. Our results, which demonstrate differences in the quality of neutralizing antibodies depending on virus serotype and pre-vaccination immune status, provide better understanding of the efficacy data from dengue vaccine trials. Samples from DENV naïve subjects at baseline were analyzed, and DENV neutralizing antibodies induced by CYD-TDV were measured. There was considerable variability in the levels of neutralizing antibodies to the different serotypes, where the mean levels to serotype 2 and 4 were higher than 1 and 3. Further analysis showed that the majority of these antibodies were cross-reactive (CR) with the exception being DENV4 which was mostly type-specific (TS) antibodies. Samples from pre-immune vaccinees, at one month post the final dose were similarly analyzed. Data shows that the neutralizing antibody titers are higher compared to the naïve recipients. The majority of these neutralizing antibodies are CR, with the highest titers to DENV1, 2 and 3. In contrast to the other serotypes DENV4 neutralizing antibodies in pre-immune individuals were at similar levels as the naïve vaccinees and mostly CR. Our depletion studies showed that CYD-TDV boosts CR antibodies in pre-immune individuals, while maintaining the proportion of TS antibodies the subject developed after natural DENV infection.

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SAFETY AND IMMUNOGENICITY OF AN AS03$_B$-ADJUVANTED DENGUE PURIFIED INACTIVATED VACCINE ADMINISTERED ON THREE SCHEDULES TO HEALTHY U.S. ADULTS

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The number of vaccine doses and their intervals influence a vaccine's immunogenicity. We evaluated the safety and immunogenicity of a candidate AS03$_B$-adjuvanted tetravalent dengue purified inactivated vaccine (DPIV) administered intramuscularly in a 0.5mL volume on 3 schedules to healthy, adult subjects 20-49 years of age in a phase 1/2, randomized, observer-blind study (NCT02421367). One hundred forty subjects were randomized to receive either 2 doses of DPIV (1µg per serotype) 1 month apart (N=35) or 3 months apart (N=70), or 3 doses of DPIV at 0, 1 and 6 months (N=35). Primary study objectives were i) to evaluate the safety and reactogenicity of DPIV+AS03$_B$ from Day 0 to Day 28 following each dose, ii) to demonstrate the added value of a third, booster dose at Month 6, based on humoral immunogenicity, and iii) to demonstrate the benefit of a longer interval between doses. Humoral immunogenicity was measured using a microneutralization (MN50) assay. The primary immunogenicity endpoints were MN50 titers to each DENV serotype for sera collected prior to Dose 1 and 28 days after the second and third dose. Primary reactogenicity and safety endpoints included the occurrence, intensity, and relationship to vaccination of solicited injection site and general adverse events (AEs) for Days 0-6 after each dose and for unsolicited AEs for Days 0-27 after each dose. Grade 2 and Grade 3 laboratory abnormalities were determined at Days 0 and 7 after each dose. Occurrence of potential immune-mediated diseases (pIMDs), medically

attended AEs, and serious adverse events (SAEs) were reported from Day 0 through Day 28 after the last dose (Month 7 Visit). The results generated in this ongoing clinical trial and their clinical implication will be presented.

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STOCHASTIC SPREAD OF WOLBACHIA THROUGH *AEDES AEGYPTI* POPULATIONS IN SPATIALLY HETEROGENEOUS LANDSCAPES

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Infection with Wolbachia bacteria suppresses *Aedes aegypti* populations and reduces their competence as a vector for dengue. Because Wolbachia-infected mosquitoes have a reproductive advantage over their wild type counterparts, widespread introduction of Wolbachia into wild *Ae. aegypti* populations is considered an efficient, long term control measure for dengue. However, the success of this strategy depends on whether Wolbachia can spread spatially through the mosquito population from a small number of initial releases. In particular, variations in habitat may slow or halt the spread. Using a two-dimensional, stochastic metapopulation model which incorporates the full dynamics of the mosquito life cycle, we investigate how spatial heterogeneities in habitat affect the likelihood and speed of Wolbachia spread. We generate clustered landscapes containing both good and bad habitat. Landscapes are classified according to the proportion of good habitat they contain and how clustered together good habitat is. We also consider landscapes in which good and bad habitat are randomly interspersed. For each landscape we simulate the release of Wolbachia mosquitoes multiple times. Overall we find that for random landscapes the speed of spread is independent of the amount of good habitat. For clustered landscapes, the speed of spread is high when the amount of good habitat is either very low or very high, but decreases for intermediate values. This reduction in speed is more prominent for highly clustered landscapes. However, the likelihood and speed of spread also depends on habitat quality in the release patch; in highly clustered landscapes with a high proportion of good landscape, released Wolbachia-infected mosquitoes will fail to invade if they are released in an island of low quality habitat. These results highlight the importance of understanding and accounting for habitat diversity when modelling and planning for large scale Wolbachia releases as a dengue control measure.

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EXPLORING THE ROLE OF ASTHMA IN DENGUE PATIENTS

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Dengue is a self-limited, systemic viral infection transmitted by mosquitoes that has a wide spectrum of clinical presentations, ranging from undifferentiated fever to severe dengue. Severe dengue can lead to serious complications and death, but if identified early morbidity and mortality can be reduced. Since epidemiologic studies had associated asthma with severe dengue, our study aims to describe demographic characteristics and clinical manifestations of laboratory confirmed dengue cases with past medical history of asthma. Data was collected from patients enrolled in the Sentinel Enhanced Dengue Surveillance System (SEDSS) established in St. Luke's Episcopal Hospitals in Ponce and Guayama, Puerto Rico from May 7, 2012 to May 6, 2015. We will compare asthmatic cases with acute, intermittent and persistent disease to determine their risk for severe dengue. SEDSS collects clinical and demographic data and specimens for testing with RT-PCR and immunodiagnostic methods as appropriate for 21 pathogens that cause acute febrile illness, including DENV. Of 1,691 enrolled patients with history of asthma, 169 had laboratory confirmed dengue. half (50.9%) of which were male ;and median age was 14.0 years (range: 1 - 73). One hundred thirty nine (77.5%) were patients under 19 years. Seventy nine cases (46.7%) were admitted to the hospital, of which 55.7% were between 10-19 years. One case was transferred to another institution (0.6%) and no deaths were reported. The most common symptoms upon presentation were headache (148, 88.1%), fever (126, 76.4%), muscle pain (114, 68.7%), facial flush (100, 59%) and rash (81, 50.3%). Other co-morbidities of asthmatic cases were diabetes (14, 8.3% and hypertension (13, 7.7%). Patients with a history of asthma and laboratory confirmed dengue had similar symptoms as dengue cases described in the medical literature. Admission rates were high for this group, therefore further analysis is being conducted to characterize and compare asthma severity among patients enrolled in SEDSS who developed severe dengue during their clinical course.

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ESTIMATION OF THE MAGNITUDE OF DENGUE INCIDENCE UNDERREPORTING THROUGH A MODELLING WITH DISMOD II SOFTWARE

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National surveillance systems have as a main goal to provide information on imminent threats to public health and to help in the monitoring of priority diseases. However, it is not uncommon to use figures coming from surveillance systems as proxies of real incidence rates. Surveillance data usually underestimates the real incidence, either because some patients might not look for care, or because symptoms may lead to misdiagnosis, as well as other organizational reasons influencing quality of report. In the specific case of dengue surveillance, the underreporting is linked to the high frequency of mild cases and the difficulty for some physicians to recognize the disease. The magnitude of the underreporting is a matter of concern, since the adequate gauge of the economic and sanitary burden of this disease, as well as the solid assessment of control measures, require robust data. Consequently, several approaches have been used to correct this issue and to obtain a better proxy of real dengue incidence. We used an IPM (incidence, prevalence, mortality) model to adjust dengue incidence estimates in order to have a better perspective of dengue epidemiology in Mexico. We used data correspondent to Yucatan, an endemic Mexican State located at the east of the country. To obtain our model, we used the DISMOD II software, developed by WHO in collaboration with Erasmus University at Rotterdam, in Netherlands. The inputs were dengue seroprevalence data of 2015, and official incidence and dengue specific mortality of the period 2010-2014. We assumed that all people infected by dengue virus stay seropositive for the rest of their life. Our results show that the real magnitude of dengue incidence is 6 times higher than the officially reported, reaching a rate of 1330 cases per 100,000 inhabitants. As an additional positive feature, this model allows to associate specific levels of incidence with predicted seroprevalence levels. We conclude that the use of DISMOD II and the IPM models in general, are useful to answer questions related to the internal consistency of epidemiology variables or to solve the lack of some parameter in order to understand dengue epidemiology.

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CHANGING CLIMATE AND TRAVEL ACTIVITY MIGHT EXACERBATE DENGUE TRANSMISSION IN TAIWAN

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Southern Taiwan has been a hotspot of Dengue Fever (DF) transmission since 1998. The incidence of dengue fever in Taiwan shows strong seasonality. Mosquito ecology and the transmission of dengue fever are influenced by multiple environmental factors, especially for climate

variations. Thus, interannual variability in climatic conditions could be important drivers for annual outbreaks. Taiwan has experienced tremendous dengue outbreaks in 2014 and 2015. Whether the sharp increase of dengue cases was due to recently climate changes or other factors should be investigated carefully. This study explored the spatial patterns of dengue outbreaks in Tainan and Kaohsiung City in Southern Taiwan throughout 1998 to 2015. Multiple climatic indices generated from weather stations and satellite remote sensing images at these two study regions were used to develop models to evaluate the impacts of changing climate on dengue transmission. Two strategies have been applied in the analysis. (1) Distributed lag non-linear model (DLNM) has been used to capture the lag effects of local climatic parameters. (2) Regional El Niño Southern Oscillation (ENSO) and Indian Ocean Dipole (IOD) effects have been evaluated by wavelet analysis. Travel statistics has been acquired to analyze the increasing patterns and their countries or origin. The Inter-annual variability of dengue outbreaks is obvious and the large-scale outbreaks might be related to a 4-year interval, however, the periodicity has been reduced after 2006. The results of DLNM have highlighted the important short effects of temperature and rainfall. However, ENSO and IOD have demonstrated different coherency patterns in the whole study period and partially explained the significant outbreaks in 2014 and 2015. Non-climatic factors, including the gas pipeline explosion in 2014 and increasing trends of tourists from endemic regions might play certain roles. Our study has revealed that dengue transmission might become more complicated due to the interaction between climate changes and human activity.

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SEROTYPE-SPECIFIC CHARACTERISTICS OF THE NEUTRALIZING ANTIBODY RESPONSE TO THE SANOFI PASTEUR DENGUE VACCINE IN PHASE III EFFICACY TRIALS

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Two Phase III efficacy trials (NCT01373281 and NCT01374516) of Sanofi Pasteur's tetravalent dengue vaccine (CYD-TDV) were conducted in dengue endemic areas of Asia-Pacific and Latin America, with subjects between the ages of 2 and 14 and between the ages of 9 and 16, respectively. The vaccine was administered in 3 doses: baseline, month 6, and month 12. The primary per-protocol analysis of vaccine efficacy was assessed for 12 months from 28 days after the third dose with hospital phase safety outcomes followed for 4 years beginning in month 25. The goal of this study is to assess characteristics of the anti-dengue neutralizing antibody (nAb) response generated in subjects in the vaccine efficacy studies outlined above. We employed a bead-based virus-depletion approach combined with a flow cytometry-based neutralization assay using U937 DC-SIGN, a known receptor for dengue virus attachment, cells to qualitatively assess whether the serum nAbs to each of the 4 dengue serotypes from these subjects were homotypic and/or heterotypic (cross-reactive). Employment of this method on post-dose 3 (PD3) samples from a clinical trial in a non-dengue-endemic region (NCT01134263) demonstrated that nAb responses to DENV1, DENV2, and DENV3 were primarily heterotypic while the responses to DENV4 were primarily homotypic. In endemic populations, vaccination has triggered a stronger and broadly cross-reactive neutralizing Ab response. Additional analysis of dengue-endemic sera from PD3 CYD-vaccinated or placebo subjects that subsequently developed a dengue case requiring hospitalization during the monitoring phase for serotype-specific homotypic and heterotypic nAbs. These nAb profiles of these samples were compared with age- and center-matched controls that did not acquire a hospitalized dengue case to determine whether the homotypic and heterotypic profile of the dengue-specific antibody responses in CYD-TDV-vaccinated and placebo-vaccinated individuals affects the likelihood of an individual acquiring a hospitalized/severe case of dengue.

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USE OF A BIOLAYER INTERFEROMETRY-BASED ASSAY TO DETERMINE ANTIBODY AFFINITY TO DENV FOLLOWING IMMUNIZATION WITH THE SANOFI PASTEUR DENGUE VACCINE IN PHASE II AND PHASE III CLINICAL TRIALS

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The goal of this study was to measure the affinity of the Sanofi Pasteur dengue vaccine-induced antibodies to each of the four serotypes, to help assess the role in protection of the quality of the antibodies induced by vaccination. A biolayer interferometry-based assay was developed using Octet RED384 (ForteBio) to determine dengue-specific antibody affinity and concentration. The method was developed and optimized using sera from naturally-infected dengue-experienced donors. An initial proof of concept study analyzed sera from adults in a phase II trial (NCT00740155) in a dengue non-endemic area which compared affinity changes after the first and second doses of Sanofi Pasteur's tetravalent dengue vaccine (CYD-TDV). In a second step, we used sera from the CYD14 and CYD15 phase III clinical efficacy trials (NCT01373281 and NCT01374516, respectively). The trials were conducted in dengue endemic regions in children between 2 and 14 years of age (CYD14, in Asia-Pacific), and 9 and 16 years of age (CYD15, in Latin America). Vaccine efficacy was assessed during an initial 25 month active phase, after which a 4-year follow-up safety study is still ongoing, called long-term follow-up (LTFU) hospital phase. Post-dose 3 sera samples from the active phase were selected from subjects that developed a confirmed dengue case that required hospitalization during the LTFU surveillance phase along with age and site-matched controls who did not have a confirmed dengue case. The difference in antibody concentration and affinity for vaccinated individuals versus placebo-treated subjects was assessed, in addition to comparing the antibody levels and binding strength between hospitalized and non-hospitalized subjects, and <9 and ≥9 year old age groups. This investigation could contribute to determining the role of antibody quality, including affinity, for protection from dengue virus infection.

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ENHANCED DENGUE SENTINEL SURVEILLANCE IN METROPOLITAN SRI LANKA: 2012 TO 2015

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Dengue poses significant disease burden in Sri Lanka. Geographic spread, incidence and severity has increased since the first reported dengue hemorrhagic fever (DHF) epidemic in 1989. Nationwide routine disease surveillance established two decades ago is based on clinical diagnosis, with infrequent laboratory confirmation. To obtain robust disease burden data, a laboratory based enhanced sentinel surveillance was established in metropolitan Colombo. Here we present study design and results of three years. Three tertiary government hospitals and 3 outpatient clinics were selected. Following informed consent patients presenting

with acute undifferentiated fever were enrolled. Blood samples were collected and tested for dengue-specific PCR, NS1, and IgM-ELISA at first presentation. Sub-set of samples were sent to Duke-NUS Singapore for quality assurance, virus isolation and serotyping. Total of 5,436 patients were enrolled from April 2012 to March 2015 with 2,058 (37.5%) as outpatients and 3,389(62.3%) as inpatients. Mean age was 20.4 years (range 1moth to 90years). Mean duration of illness at first presentation was 4 days. For inpatients and outpatients, 2,851(78.5%) and 471 (22.9%) had laboratory-confirmed dengue respectively. Mean duration of hospitalization was 4 days. Proportion of DHF in lab-confirmed hospitalized dengue cases was 20.8% and 5(0.26%) died. Serotypes 1 (86.5%) and 4 (13.5%) were the only viruses detected. Clinicians' diagnosis of dengue at the time of first presentation had a sensitivity of 95.7% and specificity of 46.1%. Dengue infection was responsible for high proportion of febrile illnesses during the study period, with co-circulation of serotypes 1 and 4. One fourth of hospitalized dengue cases in Colombo developed DHF, but the case fatality rate was low. Clinicians' judgement was associated with good sensitivity, but to enhance specificity laboratory confirmation is important.

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GENETIC VARIABILITY OF DENGUE VIRUS TYPE 2 AND CLINICAL OUTCOME DURING THE 2009-2010 EPIDEMIC OF DENGUE IN COLOMBIA

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The pathogenesis of the severe outcome in dengue is the result of multiple factors attributed to both, the host and the virus. The aim of this study was to determine the serotype associated with severity and the variation within NS4B and E genes among strains of DENV-2 isolated from patients with different outcomes in Colombia. Severe cases were defined according to a compound outcome (hypotension or bleeding) in the baseline or follow-up. The serotype was determined by RT-PCR. Phylogenetic reconstruction was performed by the Maximum Likelihood method. Single nucleotide polymorphisms (SNPs) were identified using MEGA v6.06. One hundred ninety eight patients were evaluated at baseline and follow-up. Serotype distribution was heterogeneous across outcomes with DENV-2 being predominant in complicated patients (OR 6.06, 95% CI: 2.10, 17.5). The type of infection (primary vs. secondary) was not associated with the presence of the compound outcome (OR 1.58, 95% CI 0.83, 3.00). In 54 DENV-2 sequences three distinct lineages within the Asian-American genotype were identified. Neither lineages nor NS4B or E polymorphisms were associated with clinical outcome; they rather were associated with the place of residence. In summary, the DENV-2 serotype was associated with the development of severe forms of the disease but no SNP in NS4B or E genes was identified as associated with severe outcomes.

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A DENGUE VIRUS TYPE 2-SPECIFIC MONOCLONAL ANTIBODY BINDS TO THE DENGUE VIRUS-COMPLEX-REACTIVE ANTIGENIC SITE ON ENVELOPE PROTEIN DOMAIN 3

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Dengue disease is caused by four phylogenetically and serologically-related dengue viruses (DENV) throughout the tropics and subtropics worldwide. The envelope (E) protein is the major component of the viral surface and is structurally subdivided into three domains ED1, ED2, and ED3. The majority of antibodies target ED2, and ED3 elicits potent neutralizing antibodies. Two major DENV-2 antigenic sites were previously mapped to ED3: the DENV-type specific and the DENV-complex reactive antigenic sites, which are composed of a limited subset of residues that are required for monoclonal antibody (mAb) binding. In the present study, binding to recombinant ED3 mutants and neutralization of DENV-2 strain New Guinea C mutants demonstrated that the amino acid side-chains K307, V308, K310, I312, P332, L387, L389, and N390 are functionally critical for DENV-2-type-specific mAb 9A3D-8. Surprisingly, the binding footprint of mAb 9A3D-8 is predicted to overlap primarily with the DENV-complex-reactive antigenic site on ED3. This unique binding site enabled mAb 9A3D-8 to neutralize virus infectivity at relatively low occupancy of virions compared to other mouse mAbs. Monoclonal antibody 9A3D-8 is a unique DENV-2-type-specific mAb due to its virus species specificity and its high neutralization potency, the DENV-complex reactive site for physical binding, and exhibits increased occupancy efficiency. This is a new DENV-2 type-specific antigenic site on ED3.

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PERFORMANCE OF A RAPID TEST FOR THE DETECTION OF DENGUE DURING THE OUTBREAK OF ZIKA VIRUS IN COLOMBIA

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Diagnosis of Zika virus (ZIKV) infection is done by detection of viral RNA (real time PCR) as serological tests have shown cross-reactivity with other genetically related viruses such as dengue (DENV). The aim of this study was to evaluate the performance of SD BIOLINE Dengue test for the diagnosis of DENV and to determine cross-reactivity with ZIKV in febrile patients. We evaluated 60 serum samples from patients with history of fever (less than or equal to 5 days of duration) for the detection of dengue NS1 antigen, IgM, and IgG antibodies. Thirty samples were randomly selected from participants of a dengue passive-facilitated surveillance study conducted in Piedecuesta, Colombia, before the introduction of ZIKV in the country (August to December 2014). The samples were positive for

dengue using RT-PCR and negative for ZIKV (real time RT-PCR, CDC). The Laboratory of the National Health Institute of Colombia provided a second set of 30 samples collected after the introduction of the ZIKV in the country (September of 2015), which were confirmed as positive for this virus (real time RT-PCR, CDC) and negative for DENV (real time RT-PCR, CDC). All 60 samples were tested for dengue NS1 antigen, IgM, and IgG (SD BIOLINE dengue DUO [NS1/IgM/IgG]) by the same operator and in a single batch. Two trained observers independently interpreted the results. We estimated sensitivity and specificity (95% confidence interval; 95%CI) of the NS1 antigen, IgM, and IgG. The two observers fully agreed on the interpretation of all the tests. Twenty out of 30 samples of confirmed cases of DENV were positive for NS1, that is, a sensitivity of 66.7% (95%CI: 47.2 - 82.7). There were no false positive results of the NS1 (100% specificity). Sensitivity of IgM and IgG was the same (3.3%, 95%CI: 0.1 - 17.2) and specificities were 93.3% (95% CI: 77.9 - 99.2) and 86.7% (95% CI: 69.3 - 96.2), respectively. In this study, the rapid test SD BIOLINE showed cross-reactivity for IgM and IgG antibodies but not for NS1. Further studies will test the reproducibility of our findings and control for variables such as viral serotype, duration of disease, type of infection and host genetics.

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EARLY CLINICAL INDICATORS OF DEVELOPING SEVERE DENGUE IDENTIFIED FROM A PROSPECTIVE ACUTE FEBRILE ILLNESS STUDY IN PUERTO RICO

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Because dengue is a dynamic disease characterized by progression in some to a life-threatening disease, early identification of at-risk patients could enable timelier follow-up and initiation of supportive care. To identify early clinical features associated with severe dengue, data from a prospective AFI study conducted in Puerto Rico during May 7, 2012-May 6, 2015 was analyzed. Febrile patients presenting to the emergency department were enrolled and followed through their illness. Blood and nasopharyngeal specimens were collected and tested by RT-PCR and immunodiagnostic methods for dengue viruses 1-4 and 16 other pathogens. Dengue patients who were not severe at enrollment but later developed severe dengue (Cases) were compared with dengue patients who never developed severe dengue (Controls). Of 684 laboratory-positive dengue patients with complete follow-up, 174 (25%) met criteria for severe dengue, 90 (52%) at enrollment and 84 (48%) later in their clinical course. Cases and controls were similar with regard to age, but cases were more likely to be female (62%, p = 0.003). At enrollment, cases were more likely to have anorexia (91% vs. 76%, p = 0.01), nausea (80% vs. 63%, p = 0.01), and leukopenia (87% vs. 72%, p = <0.01). Controls were more likely to present with rash (51% vs. 38%, p = 0.05) and hemoconcentration (37% vs. 17%, p = <0.01). After controlling for age and sex in logistic regression analysis, cases were more likely to have nausea at enrollment (OR = 2.70, 95% CI: 1.28-4.90) and leukopenia (OR = 2.67, 95% CI: 1.34-6.58), while controls were more likely to have rash (OR = 0.47, 95% CI: 0.28-0.80) and hemoconcentration (OR = 0.39, 95% CI: 0.19-0.78). Cases were more likely to have history of asthma (OR = 2.33, 95% CI: 1.12-3.78). Enrolling febrile patients at initial presentation enabled an unbiased determination of early clinical predictors of severe dengue. These data suggest that nausea and leukopenia may be predictors of developing severe dengue. Clinicians should be aware that patients with asthma or those presenting with nausea are at increased risk for developing severe dengue after initial presentation.

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DECODING THE SANOFI PASTEUR DENGUE VACCINE INFECTIVITY AND IMMUNOGENICITY USING THE HUMAN *IN VITRO* MIMIC® SYSTEM

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Sanofi Pasteur's licensed tetravalent dengue vaccine generates equivalent neutralizing titers against all 4 serotypes and pooled phase III clinical data in subjects over 9 years of age demonstrated conclusive, but differential levels of efficacy against the serotypes. To better understand the efficacy trial results, we studied the infectivity of the phase III lots using the innate arm of the MIMIC® system. The MIMIC system's peripheral tissue construct was adapted to assess DC viral infectivity and viral progeny levels in the supernatants for each of the 4 serotypes from the CYD vaccine as well as DC activation and cytokine profiles. The hierarchy of response for infectivity and viral progeny as measured by RT-PCR and plaque assay, respectively, for CYD vaccines in MIMIC was CYD4>CYD3>CYD1>CYD2. Previous published data and current experiments show that *in vitro* infectivity studies in IL-4 and GM-CSF derived mDCs do not show any differences between CYD serotypes, which could be due to the high level of DC-SIGN expression on the cytokine-derived mDCs. We also tested whether the difference in infectivity was due to interference between vaccine serotypes by evaluating them individually as monovalents. We show that no competitive inference was detected among the CYD serotypes in the MIMIC system and showed the same infectivity hierarchy for the monovalent lots. We assessed the immune profile induced by the CYD dengue vaccine and were able to detect up-regulation of CD86, and secretion of CXCL10/ IP-10 which have been reported as protective innate signatures in dengue infections. We also show that the pro-inflammatory cytokines such as Macrophage Inhibitory Factor (MIF) and IL-8 are secreted at markedly lower levels after infection with the dengue vaccine than by parental DENV controls. Further studies have been initiated to investigate how *in vitro* infectivity in the MIM aligns with clinical results in endemic populations following vaccination. These analyses demonstrate that the CYD dengue vaccine induces a protective innate immune signature and that the MIMIC system is a valuable preclinical tool for future vaccine candidate evaluations.

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CLINICAL IMPACT OF DENGUE AND RESPIRATORY VIRUS CO-INFECTION IN A PASSIVE SURVEILLANCE, IN THE PERUVIAN AMAZON, PERU

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Dengue virus is an important cause of morbidity and mortality in developing countries. In the Peruvian Amazon where dengue is highly endemic, respiratory viruses also have significant public health impact, especially in the cities of Iquitos and Yurimaguas. In some periods, both viruses co-circulate resulting in co-infections whose clinical characteristics remain poorly characterized. Clinic-based passive febrile surveillance established by NAMRU-6 in both cities provided the opportunity to analyze these viral co-infections. From 2010-2014, patients with acute undifferentiated febrile illness and/or influenza like-illness who sought medical evaluation in one of the thirteen hospitals or primary health

care facilities, supplied clinical data and paired blood samples and/or an oropharyngeal swab. Acute samples were tested for dengue or respiratory virus by cell culture or PCR, while acute and convalescent blood samples were tested by ELISA for dengue IgM. Of 7,150 febrile cases, 2,145 (30%), 597(8.3%), and 15 (0.2%) had laboratory evidence of dengue, respiratory virus, and co-infection, respectively. Most of the dengue infections (1616 cases, 75%), were due to serotype 2. Of the fifteen co-infections, 8 (53%) were due to Influenza B, 3 (20%) to Influenza A/H1N1pdm09, 2 (13.3%) to Influenza A/H3N2, and 2 were unidentified subtypes of Influenza A. Eleven cases (73%) were detected between February and June 2014. Rhinorrhea, cough, and shortness of breath were associated with co-infection in contrast to dengue alone ($p < 0.05$, Pearson Chi square test), while hospitalization rate (26.6 vs. 25.3%) and shock (0 vs. 0.1%) were similar in both groups ($p>0.05$). There were no fatal cases. Co-infections reported here, did not display enhanced severity compared to DENV-2 infections. Considering that asymptomatic/non-febrile cases are reported for both types of infections were not included, and despite seem to be a temporal correlation there was not a correlation spatial circulation at fine-scale foci (house). A cohort study with geo-spatial analysis would be important for continuing this work.

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ROLE OF SKIN MAST CELLS IN DENGUE VIRUS INFECTION

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Dengue virus (DENV) causes the most common vector-borne viral disease in humans living in the tropics. Transmission of DENV occurs when a mosquito vector takes a blood meal from a DENV infected host. The DENV-containing saliva is deposited in the skin of the host during probing. Human skin contains many types of immune cells, including mast cells. Here, we show that mast cells are a target of DENV in human skin and that DENV infection of skin mast cells induces degranulation. Additionally, DENV infection of primary human skin mast cells results in altered cytokine and growth factor expression profiles. Importantly, we also demonstrate for the first time that DENV localizes within secretory granules in infected skin mast cells. In addition, DENV within extracellular granules was infectious *in vitro* and *in vivo*, trafficking through lymph to draining lymph nodes in mice. We demonstrate an important role for human skin mast cells in DENV infection and identify a novel mechanism for systemic spread of DENV infection from the initial peripheral mosquito injection site. Together, these findings demonstrate a critical previously unrecognized role for skin mast cells in the infection and propagation of DENV in humans.

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DENGUE VIREMIA IN KENYAN CHILDREN WITH ACUTE FEBRILE ILLNESS

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Dengue virus (DENV) remains the most prevalent arboviral infection worldwide, causing an estimated 400 million infections per year. By mathematical modeling, 16% of DENV infections occur in Africa. However due to lack of routine surveillance programs, the burden of DENV infection in many African countries is largely unknown. As a part of an ongoing study on arboviral infection in Kenyan children, we collected blood samples from subjects 1 to 17 years of age who presented with fever of unclear etiology to one of four centers located either in western (Kisumu and Chulaimbo) or coastal Kenya (Ukunda and Msambweni). We tested the samples for the presence of DENV RNA by RT-PCR. DENV RNA positive samples were then serotyped by PCR. Testing is ongoing, however, preliminary results have identified DENV viremia more frequently in subjects with acute febrile illness in western vs. coastal Kenya (9.2% positive [75 of 814 subjects] vs. 0.9% [4 of 435 subjects], respectively, $p<0.001$). In western Kenya, all four serotypes were identified; 51 samples had serotype 1 (DENV-1), two had DENV-2, thirteen had DENV-3, and two had DENV-4. There were also subjects who had dual infection with two DENV serotypes: one subject with DENV-1+3, three with DENV-1+4, and one with DENV-2+3. Only DENV-1 was identified in samples from coastal Kenya. To investigate the phylogeny of the DENV strains, we performed exploratory next-generation sequence (NGS) on a limited subset of the samples. DENV-1 sequences were over-represented from blood samples of seven subjects from western Kenya (five from Chulaimbo, two from Kisumu) and mapped to a strain from Thailand (accession AF180818) with 98.5-99.4% homology. Further sequencing experiments will be important for developing a better understanding of the phylogeny of DENV strains currently circulating in Kenya. Together, our preliminary results suggest that DENV may be an important cause of acute febrile illness in Kenyan children, but the incidence and disease burden may differ by geographic location.

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DEFINING THE CLINICAL MANIFESTATIONS OF ZIKA AND DENGUE PATIENTS ATTENDED IN RIBEIRÃO PRETO, BRAZIL

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Zika is an emergent arthropod-borne disease that is circulating in Brazil since 2015. Since last year, we suspected that zika virus (ZIKV) was circulating in Ribeirão Preto (RP) at the same time of dengue virus (DENV). Up to this moment, little is known about the clinical aspects of this disease. Hence, the present study aimed to investigate the clinical presentation of Zika in patients with a probable diagnosis of Zika or Dengue referred to a tertiary hospital of RP. We conducted a retrospective analysis of the 162 patients attended from January to March 2016. Of these, 43 had positive results for ZIKV by RT-PCR in blood, 44 had dengue confirmed by detection of NS1 antigen and/or ELISA for IgM antibodies against DENV and 75 had other febrile illnesses. Rash was found in all Zika patients, predominantly macular or maculopapular and one had petechial rash; 57% had a malar rash. The rash usually appeared in the first day of symptoms. Among dengue patients, 75% and 37.5% had exanthematic and malar rash, respectively. Zika and dengue presented with malaise in 81.8% and 80%; myalgia in 63.0% and 78.3%, respectively. Arthralgia was more frequent in zika than in dengue (82.6% and 55.7%, respectively) although in both groups, about 20% of the patients with arthralgia had periarticular swelling or arthritis. Fever was present in 69.2% of dengue but was not common in Zika (37.9%). Conjunctival injection was reported in 59.1% of Zika and only in 28.6% of dengue patients. Less common symptoms in Zika patients were headache (52.2%), retro-orbital pain (53.3%), nausea (18.2%) or vomiting (9.1%), diarrhea (13.6%), and bleeding (4.0%). Upper respiratory tract symptoms were observed only in dengue patients (16.7%). Characterization of disease presentation is important for diagnosis to differentiate between other arboviral diseases that could occur simultaneously in the same area, especially in epidemic situation where laboratory testing become impracticable. Although there were some distinction between Zika and dengue, our data show the clinical hurdles to differentiate these diseases and justify the need for developing a fast and specific laboratory test for Zika.

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BINDING OF HUMAN MONOCLONAL ANTIBODIES TO DENGUE VIRUS WITH DIFFERENT MATURATION STATUS: A COMPARATIVE ANALYSIS

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The four serotypes of dengue virus (DENV) cause the most important arboviral disease in humans. Envelope (E) protein is the major target of neutralizing (NT) antibody. The ectodomain of E protein consists of 3 domains (DI, DII and DIII). Previous studies have shown that monoclonal antibodies (mAbs) recognizing DIII are more potent neutralizing (NT) than those recognizing fusion-loop (FL) of DII. During the maturation process, the precursor membrane (prM) protein on immature particles is inefficiently cleaved by furin, resulting in a mixture of mature, immature and partially immature virions in the culture supernatants. We studied the relationships between epitope accessibility, binding avidity and NT potency of mAbs on DENV virions produced from two cell lines with differential maturation status. To generate immature, mixed and mature virions from 293T and BHK cells, DENV1 were inoculated in the presence or absence of ammonia chloride, and furin over-expression, respectively. A virion-capture ELISA were carried out to examine the relative prM content in various particles with differential maturation status, and the binding of human anti-FL and anti-DIII mAbs. Maximum binding (Bmax) and dissociation constant (Kd) were determined (GraphPad Prism 6). NT potency, accessibility and avidity were assessed by 1/FRNT50, Bmax and 1/Kd, respectively. Regardless the sources of DENV particles, anti-DIII mAbs showed significantly lower Kd to mature virions than anti-FL mAbs, suggesting the higher binding avidity on the infectious virions may contribute to stronger NT activities of anti-DIII mAbs compared to anti-FL mAbs. Some anti-FL mAbs showed different Kd to mixed virions derived from 293T and BHK cells, which can be attributed to differential maturation status of virions from the two sources. The results on DENV virions are generally in agreement with our previous study using DENV virus-like particles and have implication for immunogen design to induce more potent NT antibodies against DENV.

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SUPERENSEMBLE FORECASTS OF DENGUE OUTBREAKS

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Dengue is a mosquito-borne viral disease prevalent in the tropics and subtropics, with an estimated 2.5 billion people at risk of transmission. In many areas with endemic dengue, disease transmission is seasonal but prone to high inter-annual variability with occasional severe epidemics. Predicting and preparing for periods of higher than average transmission is a significant public health challenge. Recent work has demonstrated that accurate forecasts of the timing and severity of disease outbreaks can be generated using a framework combining a dynamical model of disease transmission and data assimilation methods. However, because no model perfectly represents transmission dynamics in the real world, infectious disease forecasts made by a single model are prone to error due to this model misspecification. In weather and climate forecasting, this problem has been addressed by combining forecasts from multiple competing models in a superensemble. The intent is that some of the biases inherent in the different models will offset so that the superensemble produces more accurate predictions than those generated by any individual model. Here, we develop three distinct forecasting systems for dengue outbreaks in San Juan, Puerto Rico and then use Bayesian averaging methods to combine the predictions from these systems and create a superensemble forecasts. We demonstrate that on average, this approach leads to more accurate forecasts than those made from any of the individual forecasting approaches.

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LESSONS LEARNED FROM THE LARGEST AND MOST SEVERE EPIDEMIC OF DENGUE VIRUS SEROTYPE 2 (DENV-2) IN TAIWAN, 2015

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Most of dengue outbreaks in Taiwan in each year start from imported cases coming from South East Asia, except for the four over-wintering years (1987-88, 2001-02, 2009-10, 2014-15). Among all indigenous dengue cases, DENV-1 was the most common in the years before 2000, 2007, and 2014 whereas DENV-3 was isolated more frequently in 2005-06 and 2009-10 and DENV-2 was highly prevalent in 2001-03, 2011, 2013, and 2015. Therefore, DENV-2 had lower occurrence for recent 11 years until 2015. The epidemic started from dengue cluster cases occurring in the flea market of Tainan in May, 2015 and spread to Kaohsiung in July. Retrospective big data analyses identified that age groups of 19-34 and 35-49 years in Tainan played a major role of initial spreading since June 18 till July in Tainan. At the end of August, Advisory Committee was appointed by the Mayor. Health education began to target at these age groups and geographical information system (GIS) was implemented to daily evaluate emerging cases and predicting cases in future weeks, using the smallest neighborhood area (Li). By September, DENV-2 has become the dominant serotype in Kaohsiung till April of 2016, even though DENV-1 was predominant serotype from 2014 to August, 2015. Viral sequence and phylogenetic analyses of the E region found that the causing agent was the DENV-2 came from Indonesia Cosmopolitan genotype that was different from the DENV-2 in 2001-02 arisen from the Philippines Cosmopolitan genotype and the DENV-2 Asian 2 genotype in 1981 imported from the Philippines. By November, Tainan City government successfully controlled the epidemic before winter season. In conclusion, this is the first time that open-data resources and timely big data analyses were applied to be integrated with epidemiological analysis in Tainan. Most of severe dengue cases were elderly with chronic illness. Our valuable experiences indicate that web-based epidemiological information was very helpful for local residents and government officials at different levels and agencies to have rapid communication and contain the epidemic much earlier. More experiences will be shared in this meeting.

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WHAT NOW? CIRCULATION OF ZIKA, DENGUE AND CHIKUNGUNYA VIRUSES IN A CITY FROM BRAZIL

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Dengue (DENV) is undeniably a public health problem that cannot be put aside. Nor its importance can be lessened due to Zika virus (ZIKV) or any other arboviral disease. Brazil presented one of the worst outbreaks in 2015, with 1,6 million cases officially reported. There has also been an increase in disease severity and number of deaths. But *Aedes aegypti* mosquitos are not transmitting only dengue in Brazil. Chikungunya and Zika viruses are spread all over the country, including Araraquara, central portion of the State of São Paulo. The idea was to establish a potential collaboration between the Municipal Department of Health and a

research institution of the city to search for arbovirus in humans. People presenting dengue-like illness were directed to a particular Health Unity, where a trained person of our team was collecting blood and performing epidemiological surveys. We collected 362 serum samples and tested them for the presence of ZIKV, CHIKV and the four DENV serotypes by RT-PCR. DENV-1 was detected in 60 patients. DENV-4, as well as CHIKV, was detected in one patient. Eight patients were infected with ZIKV. Symptoms such as fever, arthralgia, myalgia, and rash were common. No microcephaly cases were reported in the city. The circulation of four different viruses within the urban space indicates that vector control and prevention strategies have to be reevaluated. The collaboration between two different public institutions provided epidemiological information that had never been evaluated until that point and may be an opportunity to detect virus introductions when they occur with a real possibility of avoiding viral spread.

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POTENTIAL IMPACT OF DENGUE VACCINE IMPLEMENTATION ON SURVEILLANCE AND DIAGNOSIS - INSIGHTS FROM SEROLOGICAL PROFILES OF FEBRILE CASES IN PHASE III DENGVAXIA® EFFICACY TRIALS

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Dengue surveillance is widely based on detecting IgM/IgG dengue antibodies in one post-infection sample. We previously communicated results showing that CYD-TDV vaccination induces false positive cases detected by IgM/IgG ELISA. In a post-hoc analysis of a subset of individuals from two PhIII efficacy studies (CYD14/CYD15), we describe IgM/IgG serological profile of febrile sera samples in 145 virologically confirmed dengue cases (VCD) and of 2646 febrile sera from non-confirmed dengue episodes (non-VCD) according to their dengue baseline status. This allows us to further assess the impact of dengue vaccination on serological detection of probable dengue cases IgM profile of 145 VCD cases shows that 95.9% (95%CI: 91.2-98.5) were IgM positive regardless of baseline dengue status or study group. Among 1825 febrile non-VCD episodes in seropositives, a lower but similar proportion were IgM positive in both groups (22% [19.3-24.0] in vaccine; 21% [18.0- 24.5] in control). On the other hand, among 821 non-VCD in seronegatives, a statistical difference is observed with 33% (28.7-37.0) IgM positives in vaccine and 15% (11.4-19.8) in controls, highlighting, in seronegatives, the previously reported vaccine-induced IgM positive samples. IgG profile in VCD cases indicates no difference between vaccine & control groups (100% for both in seropositives & in seronegatives 100% [89.4-100] vaccine *vs*. 89% [70.8-97.5] control). Among non-VCD episodes in seropositives, a trend for higher rate of IgG positive samples in vaccine is observed (97% [95.5-97.6] *vs*. 88% [85.6-90.8] for control). In seronegatives, a significant difference is observed (77% [73.2; 80.6] vaccine *vs*. 14% [10.2; 18.3] control; 5.5-fold difference). This highlights the impact of CYD-TDV vaccination on IgG detection in febrile non-VCD episodes. In conclusion, Dengue vaccination impacts IgM and IgG ELISA detection of probable cases in seronegative individuals. This presents a challenge for IgM/IgG ELISA based surveillance in countries where Dengue vaccine is implemented. New practical, dengue specific diagnostic algorithms are needed. * confirmed by PCRs or NS1-antigen ELISA.

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SUSCEPTIBILITY OF MEDICALLY IMPORTANT *CULEX* SPECIES MOSQUITOES TO JAPANESE ENCEPHALITIS VIRUS

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Japanese encephalitis virus (JEV) is a mosquito-borne flavivirus that is considered an important human and animal pathogen. Whilst transmission of JEV is mainly achieved by *Culex tritaeniorhychus* in endemic countries, it has been demonstrated that the virus is capable of infecting multiple mosquito species, which can subsequently become competent vectors for disease transmission. Previously, our group has demonstrated *Cx. quinquefasciatus* in the United States is a competent species for JEV. However, susceptibility of other medically important *Culex* species mosquitoes in North America remains undetermined. Based on the importance in the transmission of arboviruses in North America, susceptibility of three American *Culex* species mosquitoes to JEV was determined through oral infection. *Cx. pipiens* was chosen based on its importance in the transmission of West Nile virus (WNV) and Saint Louis encephalitis virus. *Cx. restuans* and *Cx. tarsalis* were tested because of their role as competent vectors for WNV and Western equine encephalitis virus, respectively. Infection and dissemination were observed at 7 and 14 days post infection in all three species, indicating that multiple medically important *Culex* species mosquitoes are susceptible to JEV. As observed with WNV, which rapidly established itself in the United States by utilizing multiple species of mosquitoes as vectors, JEV is a significant public health threat to the United States as it also possesses the capacity of infecting multiple species of mosquitoes. In the event of its introduction, establishment of enzootic transmission can take place by infecting multiple species of mosquitoes.

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DECREASED ZIKA VIRUS REPLICATION IN MOSQUITO CELLS CO-INFECTED WITH NHUMIRIM VIRUS

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Nhumirim virus (NHUV) is a flavivirus isolated from Brazil with an apparent insect-specific host restrictive phenotype. Previous studies in *Aedes albopictus* cells (C6/36) have demonstrated the ability of NHUV to restrict West Nile virus (WNV) growth more than a million fold, Saint Louis encephalitis virus (15,000-fold) and Japanese encephalitis virus (80-fold). Similar viral growth reduction was demonstrated for WNV/NHUV co-infection in alternative *Ae. albopictus* cells (C7-10). *Culex quinquefasciatus* and *Cx. pipiens* mosquitoes were intrathoracically inoculated with WNV or NHUV/WNV and transmission assessed. A decreased proportion of WNV transmitting Cx. *quinquefasciatus* mosquitoes was observed in NHUV/WNV mosquitoes at 7 and 9 days post-inoculation (dpi) compared to WNV-only, suggesting NHUV could act as a potential transmission barrier for other medically important flaviviruses. Currently, there are no approved vaccines or prophylaxis treatments for human Zika virus (ZIKV) infections so reduction in exposure rates to ZIKV infected mosquitoes is the principal means available to reduce human disease. To asses whether ZIKV replication is affected by pre- or concurrent NHUV infection, C6/36

cells were concurrently inoculated with NHUM (MOI 5) and ZIKV (MOI 0.1) or similarly inoculated with NHUV at 5, 3, or 1 day(s) prior to ZIKV inoculation and titers determined by plaque assay. ZIKV-only cultures achieved significantly higher titers at 1-7 dpi than dually inoculated cells. Mean peak titer of ZIKV-only cultures was 10.5 log10 (PFU/ml) while peak titers for pre- or concurrently inoculated NHUV/ZIKV cultures ranged from 3.4 - 5.5 log10 (PFU/ml), resulting in an approximate 100,000-fold reduction. No effects on timing of NHUV infection compared to ZIKV inoculation were observed. These *in vitro* results suggest NHUV could serve as a possible ZIKV transmission barrier, thus potentially modulating Zika vector competence and force of transmission in geographic areas in which NHUV and similar flaviviruses exist. Dual infection *in vivo* studies in prospective western hemisphere ZIKV vectors are being planned to address this potential.

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AVAILABILITY OF ZIKA VIRUS INFORMATION ON OBSTETRIC PRACTICE WEBSITES AND SOCIAL MEDIA ACCOUNTS IN THE UNITED STATES

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Provider to patient communication, whether domestically or internationally, is critical during a public health emergency. Understanding health care providers' utilization of the Internet for dissemination of health information is useful for emergency public health messaging strategies. In early 2016, the Centers for Disease Control and Prevention and the American College of Obstetricians and Gynecologists advised health care providers to communicate the risks of Zika virus (ZIKV) infection and prevention methods to their patients. This study's objective was to estimate the proportion of obstetric practice websites in the U.S. providing information on ZIKV within two weeks following initial release of interim guidance for care of obstetric patients. Out of 1,004 obstetric practice websites examined, only 244 (24.3%) posted information pertaining to ZIKV on their websites or website-linked social media accounts. Among those posting on ZIKV, information was more often found on their practice-sponsored social media accounts (74.2%; p=0.006) or elsewhere on their website (45.9%; p=0.35) compared to the homepage (17.2%). Practices affiliated with non-academic hospital systems and academic-hospital systems were significantly more likely to post ZIKV information (31.3%, odds ratio [OR] 2.71, 95% confidence interval [CI] 1.90-3.87; 51.3%; OR 6.29, 95% CI: 3.99-9.91, respectively) compared to obstetric practices (14.4%). 84.8% of the content posted mentioned international travel advisories and 62.3% provided information on the use of insect repellent. Although there is growing use of the Internet and social media to provide patients with health information, ZIKV information was not readily available on most obstetric care practice websites within two weeks following the release of national guidance on care of obstetric patients. Ensuring that providers around the world have the information they need to confidently utilize their practice websites and social media accounts for provision of urgent health information could keep patients as informed as possible during an evolving public health emergency.

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ZIKA AND OTHER MOSQUITO-BORNE VIRUS DETECTION AND DIFFERENTIATION USING A MULTIPLEXED, BEAD BASED RT-PCR ASSAY

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Since May 2015, South and Central America have experienced an epidemic outbreak of Zika Virus. Several travel related cases have already been identified in the United States. Zika infection during pregnancy has been implicated in the development of microcephaly in the developing fetus and is thought to be responsible for an increased incidence of Guillain-Barre syndrome. The expanding footprint of the disease, along with the devastating neurological effects associated with Zika Virus warrant rapid and early detection of Zika Virus during the critical viremic phase. In areas with co-circulating mosquito borne illnesses, viruses must not only be detected, but differentiated in order to provide accurate results. An RT-PCR based multiplex microsphere assay was developed to detect and differentiate Zika Virus, Chikungunya Virus, Dengue Virus (Serotypes 1-4), West Nile Virus, and Yellow Fever Virus. Assay performance was established using representative virus strains and clinical samples. The assay demonstrates required analytical sensitivity and is able to detect and differentiate each virus. The multiplex RT-PCR assay eliminates the need for sequential testing and complicated patient care algorithms. The multiplex format allows for simultaneous identification of viruses among a group of viruses that are difficult to distinguish due to simultaneous circulation of the viruses and strong similarities in the associated symptoms.

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ARBOVIRUS IMMUNOHISTOCHEMISTRY: CHARACTERIZING CROSS-REACTIVITIES OF DIFFERENT IMMUNOHISTOCHEMISTRY ASSAYS

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Dengue (DENV), Zika (ZIKV), yellow fever (YFV), chikungunya (CHIKV), West Nile (WNV) and Japanese encephalitis (JEV) viruses are mosquito-borne arboviruses. Several other viruses share the same *Aedes* vectors, overlap in their endemic areas, have recently re-emerged globally, and pose a major threat to public health. While RT-PCR in general is the most specific and sensitive test for these arboviruses in pathology specimens, immunohistochemistry (IHC) has value in demonstrating the presence of viral antigens in tissue and can provide insight into pathogenesis. Because serological cross-reactivity is well documented, particularly for flaviviruses, it is imperative to evaluate the level of cross-reactivity of arbovirus antibodies (Ab) used in immunohistochemical assays. In a diagnostic reference laboratory, we tested six different arbovirus Ab against cell controls, made with cells infected with different flaviviruses, and RT-PCR confirmed clinical specimens to characterize cross-reactivities. IHC assays were performed on 4µm sections of FFPE tissue using a polymer-based indirect immunoalkaline phosphatase detection system with colorimetric detection of antibody/polymer complex with Fast Red Chromogen. The JEV showed cross-reaction with all flavivirus-infected tissue tested. WNV and ZIKV assays had minimal cross reactivities with only one other arbovirus (CHIKV and DENV respectively), and YFV and DENV assays had intermediate cross-reactivities with 2-3 other arbovirus (YFV cross-reacts ZIKV and DENV, and DENV cross-reacts WNV, ZIKV and JEV). The observed assay cross-reactivity are directly related to genetic similarities among the viruses. Importantly however, different viruses have characteristic tissue

and cellular tropism by IHC which contributes to its diagnostic utility. These cross-reactivity data provide insight to the strengths and limitations of IHC assays for certain arboviruses in a diagnostic reference laboratory.

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CHARACTERIZATION OF PATIENTS WITH GUILLAIN-BARRÉ SYNDROME DURING THE ZIKA EPIDEMIC IN VENEZUELA

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Guillain-Barré syndrome (GBS) is an acute or subacute autoimmune inflammatory demyelinating polyradiculoneuropathy. It is the most common cause of flaccid paralysis and can be life threatening. GBS is characterized by a rapid-onset acute symmetrical muscle weakness, habitually ascending, affecting 1:100,000 people. In most cases GBS has been preceded in the last 6 weeks by gastroenteritis or a respiratory tract infection. During the zika virus epidemic that affects Venezuela since December 2015, a massive and rapid increase in the number of GBS patients presenting to the emergency department of the main referral hospital (CHET) of Valencia city, Venezuela has been reported. Typically, one GBS patient per month is recorded at CHET however, during the zika epidemic around 45 cases were admitted during the months of January and February 2016. We aim to characterize the clinical presentation of GBS patients and the possible association with a previous or current zika virus infection. A study is ongoing to collect clinical, laboratory and paraclinical tests data of confirmed GBS patients after informed consent along with a blood sample to perform differential diagnosis between zika, chikungunya and dengue virus infection, as these three arboviruses currently co-circulate. Preliminary results of 12 patients indicate a mean age of 56 years (range 37-83 years) with a predominance of male patients (67%). A short time (average= 6.5 days, range 1-15 days) is described between the onset of symptoms compatible with Zika infection and the beginning of neurological symptoms. Most patients rapidly progressed to life threatening disease within 24-48h. Five patients were admitted to intensive care unit of which three died (mortality=25%) despite treatment with plasmapheresis and/or immunoglobulin. A detailed description of the clinical spectrum, possible risk factors and serological diagnosis of arboviruses will be presented on the totality of patients included in the study.

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VIREMIA AND CLINICAL PRESENTATION AMONG NICARAGUAN PATIENTS WITH ZIKA VIRUS AND DENGUE VIRUS INFECTIONS

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The first autochthonous cases of Zika virus (ZIKV) were reported in Nicaragua in January 2016. ZIKV now co-circulates with dengue virus (DENV) and chikungunya virus (CHIKV) throughout the country. Although the duration of viremia in Zika fever is reportedly shorter than the duration observed in DENV infections, little quantitative viremia data has been published. In the current study, we tested acute-phase serum from sequential patients with a suspected arbovirus infection. Serum was collected as part of a national surveillance system in Nicaragua. 346 samples were tested using a single-reaction multiplex real-time RT-PCR for ZIKV, CHIKV and DENV (the ZCD assay), and viremia in ZIKV- and DENV-positive samples was quantitated. Overall, 263 (76.0%) serum samples were positive for one or more pathogens. 75 patients (28.5%) tested positive for ZIKV (47 mono-infections, 28 co-infections). 109 (41.4%) patients were positive for DENV (54 mono-infections, 55 co-infections). Identified co-infections included all combinations of the 3 viruses: ZIKV-CHIKV (n=16), ZIKV-DENV (n=6), DENV-CHIKV (n=43), and ZIKV-CHIKV-DENV (n=6). The mean duration of symptoms was similar for ZIKV-positive (3.4 days, SD 1.1) and DENV-positive patients (3.4 days, SD 1.3; p=0.81). Quantitated viremia in ZIKV-positive samples (mean 4.7 log10 copies/mL serum, SD 1.0) was significantly lower than DENV-positive samples (5.8 log10 copies/mL serum, SD 1.8; p<0.01). The distribution in viremia also differed significantly (p<0.01 by Kruskal-Wallis test), and only 39/75 (52.0%) ZIKV-positive samples had quantifiable viremia compared to 94/109 (86.2%) DENV-positive samples. For both viruses, viremia was significantly lower in co-infections than mono-infected samples. Finally, an analysis of clinical signs and symptoms in relation to viremia at presentation will be presented. Results from this study have important implications for ZIKV diagnosis in the acute phase and expand upon the available literature regarding viremia in ZIKV infected patients.

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EFFECTS OF TEMPERATURE ON ZIKA, DENGUE AND CHIKUNGUNYA TRANSMISSION BY *AEDES AEGYPTI* AND *AE. ALBOPICTUS*

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The geographic distribution of vector-borne diseases is shaped by many ecological and evolutionary factors, including the response of vectors and pathogens to environmental drivers. Mosquito-transmitted diseases such as Zika, dengue, and chikungunya are intimately linked with environmental temperature and humidity because of mosquito and pathogen physiological responses, including growth, development, survival,

reproduction, and behavior. Current models often inaccurately predict that warmer temperatures will tend to increase mosquito transmission even as temperatures warm above 30°C. In contrast, models that include more physiologically accurate, nonlinear thermal responses of the mosquito and pathogen vital rates that drive transmission predict intermediate optimal temperatures. Here, we develop a model of arbovirus transmission (particularly dengue, chikungunya, and Zika viruses) by *Aedes aegypti* and *Ae. albopictus* mosquitoes that includes physiologically accurate, nonlinear mosquito and parasite thermal responses. *Ae. aegypti* and *Ae. albopictus* development rates, longevity, fecundity, and biting rates, as well as dengue virus vector competence and extrinsic incubation rates have hump-shaped responses to temperature with intermediate optima. As a result, dengue, chikungunya, and Zika virus transmission are optimal at intermediate temperatures (27-29°C) and decline steeply above 32-36°C and below 15-17°C). The model predictions are consistent with field data from the Americas on the number of human dengue, Zika, and chikungunya cases across space and time. These intermediate optimal temperatures are robust to uncertainty in trait thermal responses. We quantify sources of uncertainty in transmission across temperatures and make prescriptions for future experimental work to resolve this uncertainty. Together, the results imply that much of tropical, sub-tropical, and temperate North, Central, and South America and the Caribbean are suitable for seasonal or year-round transmission.

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GUILLAIN-BARRÉ SYNDROME RISK AMONG INDIVIDUALS INFECTED WITH ZIKA VIRUS

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As of early April, 2016, local Zika virus (ZIKV) transmission had been confirmed in 33 countries in the Americas where transmission had never previously been reported, and 9 of those countries had reported notable increases in the number of cases of the neurological disorder Guillain Barré Syndrome (GBS). These increases were spatio-temporally correlated with reports of Zika cases and ZIKV infection has been confirmed in some of the GBS cases. A potential connection between ZIKV infection and GBS was first noted during an outbreak of Zika in French Polynesia. It was later shown that all forty-two reported GBS cases in French Polynesia had evidence of previous ZIKV infection, compared to only approximately half of non-GBS controls. Estimated GBS risk in French Polynesia was approximately 2.4 cases per 10,000 ZIKV infections, more than 10 times the expected baseline risk. We built an inference framework to combine data on ZIKV infections, Zika cases, and GBS cases from locations with previous outbreaks (Yap and French Polynesia) with real-time Zika and GBS case data from the ongoing outbreak in the Americas to estimate the risk of GBS that may be associated with ZIKV infection. These estimates indicated that ZIKV-associated GBS risk in the ongoing outbreak may be similar to the risk estimated in French Polynesia, though current point estimates are slightly lower. Sensitivity analyses highlighted how integrating data from previous, better described outbreaks with current estimates can help improve confidence in estimating current risks. These methods and the estimates they produce are of paramount importance for response and preparedness planning in locations experiencing Zika outbreaks .

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RELATIVE FITNESS OF ZIKA VIRUS LINEAGES IN MOSQUITOES AND CELLS

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Zika virus has undergone a dramatic range expansion in recent years, including a dramatic outbreak that is currently ongoing in South America. Interestingly, it is likely that ZIKV, for the first time in its evolutionary history, is replicating extensively in human beings. In addition, a hallmark of arbovirus introductions into new ecological niches has been increased fitness in local vector populations. This has been clearly observed with WNV and CHIKV as they have colonized new regions. It seems likely that this may also occur as ZIKV spreads within the Americas. Therefore, we assessed the relative fitness of Asian lineage ZIKV from the new world compared to West African (Senegal 41525) and East African (Uganda MR766) lineage viruses. Briefly viruses were mixed 1:1 and allowed to compete in several test systems including: Mexican *Ae. aegypti* mosquitoes, US *Ae. albopictus* mosquitoes, US Cx. *quinquefasciatus* mosquitoes, BHK-21 cells, and human neuroblastoma, dorsal root ganglion and CRL-1973 (pluripotent testicular) cells. After an appropriate period of competition, virus was harvested from mosquitoes and relevant tissue cultures and the proportion of each competitor determined by analysis of sequence chromatograms (i.e. "quantitative sequencing.) Results of these competition studies, and their implications for virus adaptation, will be discussed.

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EVALUATING THE EFFECTS OF TEMPERATURE VARIATION ON ARBOVIRUSES

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Emerging and re-emerging mosquito-borne diseases represent a substantial global health burden. Viruses like Chikungunya, Zika and Dengue are all transmitted by the *Aedes aegypti* and *Ae. albopictus* mosquitoes. The U.S. is home to both mosquito vectors with *Ae. aegypti* constrained to highly urban environments in southern coastal states and A. albopictus distributed throughout urban, suburban, and rural areas in 26 states. This study investigates the effects of temperature on Chikungunya virus (CHIKV) and Zika virus (ZIKV) growth dynamics in mosquito tissue culture cells derived from both mosquito hosts. Aag2 (*Ae. aegypti*), C6/36 (*Ae. albopictus*) and U4.4 (*Ae. albopictus*) mosquito larval cell lines were inoculated with attenuated CHIKV (vaccine strain 181/25) and ZIKV (MEX 1-44). Samples maintained at six constant temperatures (16°C, 24°C, 28°C, 30°C, 32°C and 34°C) were collected at different time points for att. CHIKV and ZIKV. Viral titer was calculated as plaque forming units (PFUs) per milliliter through standard plaque assays on Vero cells. As expected from the literature, Aag2, C6/36 and U4.4 cell lines had differential growth responses to temperature. Cell lines grew slower at the lowest temperature and displayed slower viral replication. At the higher temperature conditions, virus growth was reduced, but to differential effect between the cell lines. These data suggest that there may be differences in viral growth kinetics at different temperatures in the two vector hosts (*Ae. aegypti* and *Ae. albopictus*) and future work will investigate these interactions *in vivo*.

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ZIKA VIRUS INFECTIONS: PAYING ATTENTION TO ATYPICAL PRESENTATIONS

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Zika disease has reemerged recently and its most common clinical manifestations are still being described. Low-grade fever, pruritic rash, conjunctivitis, arthralgia, myalgia and other minor constitutional symptoms are reported more often. Brazil has been experiencing an important Zika outbreak since May 2015, and since the beginning of this year a great number of patients was attended in our teaching hospital due to a Zika-like disease. Interestingly, patients sought medical attention primarily because the presence of a rash and not because they were feeling ill. Most patients presented with the symptoms described above and had an uneventful course; there was not a single case of microcephaly, but an increased number of Guillain-Barré Syndrome was admitted to our ICUs. Additionally, we report here three Zika confirmed cases of patients (2 males and a female) who had an atypical disease presentation. One of the patients presented with an orchiepididymitis about five weeks after an acute Zika presentation. Real-time RT-PCR was positive for Zika virus in blood and urine in both occasions. The patient recovered completely with standard treatment and remains asymptomatic. The other patient presented with muscle pain and Zika classical symptoms, but his creatine phosphokinase level (CPK) was initially 13,500 µg/L. Patient was treated for rhabdomyolysis, CPK levels reached normal levels in a week and he recovered without any renal sequelae. Finally, a young woman was referred to our hospital due to an extensive petechial rash and arthritis on knees and ankles. Lab results were all normal and prednisone was prescribed to the patient. Five days later, she was able to walk without pain and the petechial rash had faded. Real-time RT-PCR was also positive for Zika virus in blood and urine for both patients. Although all three patients recovered completely, if they were not promptly diagnosed and treated, they could have had a complicated course of the disease. Thus, although the great majority of patients will recover from Zika, we must pay attention to details of disease presentation to detect the atypical findings of this disease.

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MODEL-BASED PROJECTIONS OF ZIKA VIRUS INFECTIONS IN CHILDBEARING WOMEN IN THE AMERICAS

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Zika virus is a mosquito-borne pathogen that is rapidly spreading across the Americas. Due to a probable association between Zika virus infection and a congenital neurological disorder called microcephaly, the epidemic trajectory of this viral infection poses a significant concern for the nearly 15 million children born in the Americas each year. The potential magnitude of the ongoing Zika epidemic is exceedingly difficult to gauge based on existing data, due to a number of uncertainties that cloud the relationship between observed cases and true infections. As an alternative to methods that depend on unreliable case data, we developed and applied a new method that leverages highly spatially resolved data about drivers of Zika transmission to project that 1-2 million infections in childbearing women and approximately 100 million infections across all demographic strata could occur before the first wave of the epidemic concludes. Our projection is largely consistent with annual, region-wide estimates of 53.8 (40.0–71.8) million infections by dengue virus, which has many similarities to Zika. Based on independent estimates of microcephaly rates, our results suggest that the current epidemic has the potential to negatively impact tens of thousands of pregnancies. Uncertainties in these projections and up-to-date comparisons against case reports will be discussed.

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EVALUATING STRAIN SPECIFICITY OF THE ZIKA VIRUS NEUTRALIZING ANTIBODY RESPONSE

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Zika virus (ZIKV) is a mosquito-borne flavivirus that has emerged as a significant global health problem. Recent outbreaks of ZIKV infection in French Polynesia and Brazil have been associated with more severe manifestations, including microcephaly and intrauterine growth retardation in fetuses and infants born to women infected with the virus while pregnant. There are two lineages of ZIKV, African and Asian, that share ≥ 95% amino acid identity. The spread of ZIKV to the Americas has been attributed to the Asian lineage. No vaccines are available for use in humans, and little is known regarding the ability of neutralizing antibodies elicited against one lineage to protect against another. The primary target of neutralizing antibodies is the envelope (E) protein, of which 180 copies comprise the ZIKV virion surface. To facilitate the study of ZIKV antibody responses, we generated pseudo-infectious ZIKV reporter virus particles (RVPs) by co-transfection of a DNA-launched West Nile virus sub-genomic replicon that expresses GFP with a second plasmid encoding the ZIKV structural genes (capsid, pre-membrane, and E). ZIKV RVPs representing both African and Asian strains were used to assess neutralizing antibody responses in a panel of ZIKV-confirmed convalescent human serum samples collected 3-12 weeks post onset of symptoms. Our results demonstrated minimal strain-specific differences in neutralizing antibody responses. These findings were confirmed using a flow cytometry-based infectious Zika virus assay; comparisons of antibody titers obtained using ZIKV RVPs and fully infectious virus revealed excellent agreement. These findings suggest that vaccination with a single ZIKV strain may elicit cross-protective neutralizing antibody responses against both lineages. Furthermore, our studies establish ZIKV RVPs as a high-throughput and quantitative alternative to the use of fully infectious virus for assessing ZIKV-specific antibody responses.

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DURATION OF INTESTINAL IMMUNITY FOLLOWING ADMINISTRATION OF INACTIVATED POLIO VACCINE (IPV) IN INDIAN CHILDREN PRIMED WITH ORAL POLIO VACCINE (OPV)

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As per "The Polio Eradication and Endgame Strategic Plan 2013-2018", IPV has been introduced in all OPV using countries since the last quarter of 2015. Few studies have demonstrated boosting of short term mucosal immunity after IPV administration in children already primed with OPV, but no study has evaluated the duration of intestinal immunity after IPV administration. The aim of the study was to compare intestinal immunity to poliovirus, in OPV immunized children, 6 months and 12 months after a single supplemental dose of IPV to children who did not receive a supplementary IPV dose, measured by shedding of Sabin types 1 and 3 poliovirus in stool samples collected 7 days after a "challenge" dose

of type 1 and 3 bivalent OPV (bOPV). It was a single centre, open-label randomized controlled trial (CTRI/2014/09/004979) conducted in Vellore, India, with 3 groups enrolling a total of 900 children aged 12-59 months (300 each in control, IPV-6 month, and IPV-12 month arms). Blood samples were collected at recruitment from the control group and 28 days after IPV administration in the IPV-6 and IPV-12 groups, to evaluate anti-poliovirus neutralizing antibodies against all three poliovirus serotypes (PV1, PV2, PV3). Shedding of Sabin poliovirus 1 and 3 were determined by one-step multiplex real-time PCR. The geometric mean titres (GMT) of neutralizing antibodies against PV1, PV2, and PV3 respectively were: control arm (103.14, 186.64, 53.01), IPV-6 arm (737.34, 847.68, 839.55), IPV-12 arm (833.08, 910.61, 851.06). The proportion shedding Sabin1 poliovirus 7 days after challenge in the three arms were: control (22.3%,66/296), IPV-6 (15.8%,45/284), IPV-12 (17.8%,53/297). For Sabin3, the proportion shedding were: control (25%,74/296), IPV-6 (15.1%,43/284), IPV-12 (13.8%,41/297). There was a significant reduction in shedding of Sabin3 in the IPV-6 (p=0.004) and IPV-12 (p=<0.001) groups compared to controls. For Sabin1, shedding was significantly less in the IPV-6 (p=0.048) but not in the IPV-12 (p=0.17) group compared to controls. Thus, the study demonstrated effective long term intestinal immunity after IPV administration in OPV primed children.

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EVALUATION OF A MEASLES ROUTINE IMMUNIZATION PROGRAM IN THE DEMOCRATIC REPUBLIC OF CONGO

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Estimates of population-immunity to vaccine preventable diseases (VPDs) are useful to assess the performance of immunization programs, identify susceptible groups and at-risk populations. A comparison of seroprevalence and coverage data can be useful in quantifying the impact immunization programs have on reducing vaccine-preventable diseases such as measles. In collaboration with the 2013-14 Demographics and Health Survey (DHS) conducted in the Democratic Republic of the Congo (DRC), we evaluated the seroprevalence of IgG antibodies to measles using a multiplex format (M2) from dried blood spots from children 6 - 59 months of age and compared these results to 2013 administrative vaccination coverage data provided by the DRC Expanded Programme on Immunization (EPI) to evaluate the DRC Routine Immunization (RI) program. The lowest seropositivity rates were seen in Kasai Occidental (53.6%), Kasai-Oriental (51.9%), Katanga (51.4%), and Maniema (51.9%) provinces. Average measles vaccination coverage rates were 92%, 91%, 93.3%, 88% respectively. Of the 516 health zones in DRC, 71 reported vaccination coverage rates above 100%. Our findings suggest that gaps in measles immunity exist throughout the country and administrative coverage rates may be overestimated. Vaccine effectiveness and vaccination coverage rates should be thoroughly assessed to understand the drivers of immunity gaps that should be addressed to improve the RI system.

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DETECTION OF HUMAN ENTEROVIRUS 71 FROM AN OUTBREAK OF HAND FOOT AND MOUTH DISEASE IN BANJARMASIN, INDONESIA

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Enterovirus 71 (EV71), a single-stranded RNA virus of the Picornavirus family, is a common cause of Hand, Foot and Mouth Disease (HFMD). The infection usually manifests with fever, rash, blister and in severe cases may proceed to encephalitis. EV71 epidemics have occurred in the Asia-Pacific region, including Taiwan, mainland China, Hong Kong, Malaysia, Vietnam, Singapore, and Thailand for the past decade. We identified EV71 virus in 9 specimens from children presenting with fever and HFMD in Banjarmasin, South Kalimantan (n=13) in early 2016. Viral RNA was isolated from nasal swab, followed by cDNA synthesis using random primers. Specimens were tested by conventional PCR using enterovirus genus-level primers, followed by specific primers to determine the strain using DNA sequencing. Sequencing analysis demonstrated 98-100% similarity with the Malaysian strain and was shown to belong to subgenotype B5. Phylogenetic analysis of the VP1 gene suggests that the EV71 strain causing the outbreak in Banjarmasin could have originated from Malaysia. In parallel, virus was isolated in Green Monkey Kidney Epithelial Cells (Vero 81) which displayed severe cytophathic effects (n=7) after 2 passages. We report the finding of EV71 as a causative agent of HFMD outbreak in Banjarmasin. To our knowledge, this is the first detection and isolation of EV71 in Indonesia.

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DYNAMIC MODEL OF ROTAVIRUS TRANSMISSION WITH IMPACT OF TEMPERATURE

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Although rotavirus exhibits seasonality, water quality risk assessments do not consider temperature. Additionally, prior rotavirus transmission models have not previously considered the water as a driver of transmission. We conducted a meta-analysis to estimate the effect of temperature on rotavirus decay rate separately for monophasic and biphasic decay. We then used these estimates in a rotavirus transmission model that explicitly modelled the water reservoir. The model was seeded by allowing water to flow into the water reservoir from an upstream community. We assessed the critical transmissibility from water to humans necessary to seed the outbreak and assessed how this might vary by temperature. Temperature was significantly associated with decay rates for monophasic decay, with 8.77°C increasing in temperature related to 1 log increase in rotavirus decay rate ($p = 0.0003$). For biphasic decay, temperature had a marginally significant effect on the first phase of decay with 3.37°C being associated with a 1 log increase in rotavirus decay rate ($p = 0.0735$). Temperature did not appear to effect the second phase of decay. In our transmission model, we found that a rotavirus outbreak was inevitable as long as pathogen existed in water source regardless of the transmission rate from water to humans, because of the combination of high shedding rates and low infectious dose for rotavirus. Temperature altered the time period before peak of infected arrived, with higher temperatures leading to slower outbreaks. These results show a mechanism by which temperature may affect risk of rotavirus and suggest that the water may be an effective conduit of disease between communities.

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FIRST LABORATORY CONFIRMATION OF AN OUTBREAK OF RIFT VALLEY FEVER VIRUS IN 50 YEARS IN KABALE DISTRICT, SOUTHWESTERN UGANDA

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On March 10, 2016, the Uganda Virus Research Institute/Centers for Disease Control (UVRI/CDC) Viral Hemorrhagic Fever (VHF) laboratory was notified of 2 suspect VHF cases from Kabale district, South Western Uganda. Both cases were confirmed as RVF by RT-PCR and IgM serology. Within 24 hours a team from UVRI, the Uganda MOH, and CDC-Uganda traveled to Kabale to carry out epidemiological and ecological investigations. Both cases presented with febrile illness and reported fever, vomiting, fatigue, abdominal pain, headache, epistaxis, and melena. The initial case was a butcher who worked in the central Kabale abattoir. The second case was a student who resided approx. 12Km south from central Kabale. The two cases were not epidemiologically linked. There were a total of 8 suspect cases and 2 probable deaths identified. The team performed an initial investigation to determine the extent of the outbreak. Samples from 21 family member and community members of the confirmed and probable cases were collected, along with 86 livestock specimens from the same locations. One suspect human case was positive for RVF by IgG, but negative for IgM and RT-PCR, and classified as a convalescent confirmed case. No additional human cases were confirmed from the samples collected. 9% of livestock specimens tested positive for RVF by IgG, and one caprine from the village of one of the confirmed cases also tested positive by RT-PCR. An expanded district-wide human and livestock serosurvey was initiated following these results to determine how widespread RVF transmission is in the region. A total of 657 human and 1052 livestock samples were collected and are currently being tested. Extensive outbreaks of RVF have occurred elsewhere in East Africa, notably in 1997-8 and 2006-7. This RVF outbreak in Kabale represents the first reported laboratory confirmed human cases in Uganda since 1968. It also represents the 11th independent VHF outbreak confirmation in Uganda since the beginning of enhanced VHF surveillance in 2011.

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A RETROSPECTIVE COHORT STUDY OF SEROPREVALENCE OF EBOLA AND MARBURG VIRUSES IN HUMANS FROM TWO DIFFERENT ECOLOGICAL ZONES IN UGANDA

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Filoviruses cause high morbidity and mortality and pose a threat to human and animal populations. Uganda has experienced eight filovirus outbreaks in the past 15 years; five caused by Ebola virus and three by Marburg virus. The aim of this study was to determine if people living in and around bat inhabited mines in medium-high mountainous areas in Western Uganda might have increased exposure to filovirus infection compared to those in wooded savannah in Central Uganda. A retrospective cohort study was conducted. The exposed groups were miners and their family members; and communities within 50km from Kitaka mine in Ibanda district, Western Uganda. The control group was located in Central Uganda with no mining activity. A risk factor questionnaire was administered and blood samples collected from 740 people. Blood samples were tested for the presence IgG against Sudan Ebolavirus and Marburg virus at the Uganda Virus Research Institute. The exposed group comprised 60% (444/740) and controls were 40% (296/740). Filovirus seroprevalence was 3.2% (24/740) overall; Sudan ebolavirus 2.4% (18/740) and Marburg virus 0.8% (6/740). Although not statistically significant, the exposed group had 2.5 times the risk of being infected with filovirus compared to the control group; RR=2.5(95%CI, 0.96 - 6.7) whereas miners or their family members were 2.2 times as likely to be infected compared to other occupations (RR=2.2, 0.98 - 4.7). Other risk factors include going into mines or caves inhabited by fruit bats (RR=2.5, 1.12 - 5.66), residing in a village that had a previous outbreak of filovirus (RR=2.7, 1.24 - 6.04) and having had contact with a suspect case of filovirus infection (RR=4.5, 1.66 - 12.56). This sero-survey in Uganda indicates there may be an underestimate of filovirus infections occurring, thus more outbreaks going undetected. Health facilities need to increase their level of suspicion for Uganda viral hemorrhagic fever (VHF) as a possible cause of acute febrile illness. The Uganda VHF surveillance program has initiated enhanced febrile illness surveillance strategies to detect and respond to potential sub-clinical filovirus infections.

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THE POTENTIAL ECONOMIC IMPACT OF THE ZIKA VIRUS

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The sudden rise of Zika cases is unnerving. This situation has forced the World Health Organization (WHO) to declare the epidemic of microcephaly cases in regions affected by Zika virus a matter of public health emergency of international concern. Beyond the silent suffering among those directly affected by the Zika virus, there are many concerns about the negative impact that the Zika outbreak will have on the economy of countries affected by Zika. One concern is the potential negative impact of the Zika virus on the tourism industry. Though currently there are no travel restrictions imposed by the WHO, there have been anecdotal reports about international airlines already cancelling or rescheduling flights for passengers that are traveling to the region that are pregnant or may become pregnant. Moreover, evidence is emerging on the impact that *Aedes aegypti* diseases have on tourism revenues. Added to the potential significant losses associated with tourism, the possible decline on foreign direct investment due to the Zika outbreak is a major concern. Investments in outbreak control and surveillance infrastructure may also be impacted by the recent Zika outbreak. The loss of productivity due to the Zika outbreak is an even greater concern. At a macro level, the Zika outbreak could have other long-term repercussions. There are several challenges that affected communities in the Americas face to contain the spread of the virus. In the current systematic review we will explore these challenges and delve deep into the potential economic impact of the Zika virus. Evidence of the economic potential of the Zika virus is critical to informing policy decisions and securing financial support for future Zika prevention and control strategies.

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THE EVOLUTION AND DIVERSITY OF HUMAN METAPNEUMOVIRUS IN PERU

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Human metapneumovirus (HMPV) causes substantial morbidity in the very young, elderly and immunosuppressed, yet there is limited knowledge

of the diversity and epidemiology of this virus in Peru and other tropical countries. Therefore, we examined the evolution and spatial patterns of HMPV in Peru through phylogenetic analysis of 61 whole-genome sequences of HMPV collected in three regions of Peru; the largest genomic dataset from any tropical country to date. 61 HMPV PCR positive respiratory specimens archived from an influenza-like-illness sentinel and active surveillance study in Lima (temperate capital), Piura (northern coastal desert) and Loreto (tropical Amazon) from 2008-2012 underwent high-throughput whole genome sequencing using Illumina and Ion Torrent platforms. A 375 F-gene sequence dataset was constructed that included Peruvian and other global background sequences available from GenBank. A Maximum Likelihood tree, with 100 bootstrap replicates, was inferred by the RAxML package using a GTR + gamma nucleotide substitution model. Extensive genetic diversity of HMPV was identified in Peru, including the A2, B1 and B2 subgroups (A1 was not identified). Within a single year, the genetic diversity of HMPV identified in Peru represented nearly the entire global diversity of HMPV, owing primarily to multiple independent viral introductions into Peru each year. Possible multi-year persistence of HMPV was observed in Loreto and Piura, although low background sampling from other countries and locations in Peru complicates conclusions about local persistence. The high diversity observed in more isolated tropical locales such as Loreto and Piura is attributable to both viral introductions from other countries as well as gene flow within Peru. There is evidence of substantial HMPV viral traffic within Peru and between Peru and other global regions. Significant HMPV diversity exists even in more isolated regions of Peru. These findings underscore the rapid and extensive diffusion of respiratory viruses in tropical countries, which may have implications for the effectiveness of a HMPV vaccine across diverse global regions.

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EVALUATION OF THE BIOJECT NEEDLE FREE VACCINE DELIVERY DEVICE FOR VACCINATING RATS WITH RIFT VALLEY FEVER VACCINE CANDIDATES

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Rift Valley Fever virus (RVFV) is a major public health and agricultural concern in Africa and the neighboring region. The only potential approach for preventing epidemics of RVFV is to develop and deliver an effective vaccine for livestock and humans. The aim of this study was to evaluate a Bioject needle free vaccine delivery device for potential use to vaccinate animals. In this study as preliminary assessment the Bioject device were conducted in laboratory Wistar rats, using the device with and without a spacer, suggesting a route of delivery to be intradermal and intramuscular respectively. Two doses of the RVF MP-12NSm-del vaccine of 1 x 10^3 and 1 x 10^5 plaque forming units (PFU) were used. Blood samples were collected on day -1 before being vaccinated and at days 7, 11, 15, and 25 post-vaccination (PV), and tested for IgG antibodies by ELISA, and using 80% reduction in the MP-12 virus dose as the endpoint for determining the neutralizing antibody (NA) titers. Most all animals vaccinated with the RVF MP-12NSm-del vaccine with or without a spacer developed detectable NA by day 7 PV and persisted at or above the titers on day 7 through 25 days PV or the duration of the experiment, with titers ranging from 1:10 to 1:1280. In contrast, the ELISA optical density (OD) values were below IgG antibody positive levels on day 7 PV and not until day 11 PV that positive OD values started to become detectable, with the OD values being higher for the animals that were vaccinated without the spacer. In contrast to the animals vaccinated with the Bioject device, the NA titers and the ELISA OD values were lower for the animals vaccinated with 1 x 10^5 PFU of the RVF MP-12NSm-del and 1 x 10^5 PFU of the RVF MP-12 vaccines using a needle, thus suggesting that the Bioject needle free delivery device may be a more convenient and effective method of vaccinating animals with RVF vaccines. Finally, these preliminary data are very exciting and promising in regard to our goal of utilizing a needle free delivery method for vaccinating animals with RVF vaccines in Africa. Acknowledgement/Disclaimer: This work was made possible by the generous support of the American people through the United States Agency for International Development (USAID) *DISCLAIMER The author's views expressed in this publication do not necessarily reflect the views of the United States Agency for International Development or the United States Government.*

1395

CHARACTERIZATION AND IMAGING OF THE PRIMATE MODEL FOR NIPAH VIRUS INFECTION

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Nipah virus (NiV) is a zoonotic pathogen endemic to parts of Indonesia and the Asian subcontinent, particularly Bangladesh. Infection in humans can lead to severe respiratory or neurologic disease and death. Mechanisms of NiV-related disease and discriminators between respiratory or neurologic disease are unknown. Here, we focused on expanding the understanding of NiV infection in African Green Monkeys (AGM) by quantifying multiple parameters of the immune response to NiV infection. We also used Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) to assess disease progression following intratracheal (IT) and aerosol administration of virus. Animals developed a primarily respiratory disease, with those infected by the IT route developing pulmonary lesions with significant edema and consolidation. Animals infected by small particle aerosol developed a more diffuse disease. Two animals developed changes in the brain vasculature with suggestion of slightly engorged cortical veins late in the disease process, of unknown clinical significance. Survival times post-challenge were equal in the two groups (~8 days). Five of 6 animals developed a profound thrombocytopenia, manifested by severe subcutaneous hemorrhage at necropsy. Assessment of leukocytes revealed evidence of lymphopenia and neutrophilia, but with apparent expansion of activated CD8+ T cell populations in two of the aerosol exposed animals. These findings correlated with increases in serum cytokine levels indicating a proinflammatory response and Th1 differentiation of T cell populations. Populations of immune cells in the lungs were largely unremarkable except for elevated monocyte populations in the aerosol group relative to the IT inoculated group. These data demonstrate that IT and small particle aerosol inoculation of AGM results in a rapidly progressing respiratory disease largely devoid of neurological indications. These studies support previous work demonstrating a significant role for endothelial cell dysfunction in disease. It was evident that the rate of disease progression limited development of an effective immune response.

1396

SEROEPIDEMIOLOGICAL STUDIES FOR INFECTIONS BY VECTOR-BORNE AND ZOONOTIC PATHOGENS AMONG U.S. MILITARY PERSONNEL

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Vector-borne and zoonotic pathogens have long detracted from operational readiness of U.S. forces stateside and deployed around the world. Notable historical examples include the impact of malaria and dengue during World War II, hantavirus infections during the Korean War, and more recently, a 2003 outbreak of *Plasmodium falciparum* among marines deployed to Liberia. Military populations present a challenge for public health surveillance, as they are highly mobile and often deploy to austere settings in remote and, at times, unstable regions of the world, which limits available diagnostic and treatment options. Vector-borne and zoonotic infections are often associated with acute undifferentiated febrile

illness and thus are difficult to distinguish clinically, further exacerbating challenges for diagnosis and control. As a result, the burden of vector-borne diseases among U.S. military personnel remains poorly defined. To address this gap, we initiated seroepidemiological studies among U.S. military personnel, capitalizing on serum samples available through the Department of Defense Serum Repository (DoDSR). The DoDSR has been utilized to measure infections among DoD personnel to a diverse set of pathogens, including *Rickettsia* spp. in South Korea, Coxiella burnetii in Iraq, and *Plasmodium falciparum* in Liberia. Recently initiated studies will address infections by vector-borne and zoonotic pathogens among DoD personnel deployed to West Africa and to measure the incidence of infections by emerging viral pathogens, including dengue virus, chikungunya virus, and Zika virus, in the Caribbean. Results from these analyses, in conjunction with associated febrile illness data reported through electronic surveillance systems, can be used to inform force health protection measures and prioritize the development of diagnostics and medical countermeasures.

1397

DETERMINATION OF HEPATITIS B VIRUS (HBV) INFECTION IN FAMILY MEMBERS OF HBSAG CARRIERS: SEARCH STRATEGY OF CARRIERS FOR A ELIMINATION PLAN OF HBV

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Huanta was a city with a high endemic of hepatitis B virus (HBV). However, now a HBV elimination plan HBV is feasible because the immunization in people under the age of 20 that started in 1994 have had a good impact. The aim of our study is to determine the prevalence of hepatitis b virus (HBV) infection in family members of HBsAg carriers as a search strategy of carriers for an HBV elemination plans. This cross-sectional study included the family members (n=40) of 15 HbsAg (+) patients at Huanta's Hospital. Demographic data, vaccination status, relationship with the patient and risk factors were reviewed by a questionnaire. Blood samples were taken to determine HBV and hepatitis D virus (HDV) markers. Data were analyzed using t-test and chi-square. The protocol of this study was approved by the Ethics Committee of the Institute of Tropical Medicine of the National University of San Marcos. The 5.5% of the family members have an acute infection and 22.2% were chronically infected. All of them were over the age of 23. Family members that have or had HBV infection were significantly older, consumed significantly more alcohol and traveled significantly more to endemic zones than those who never had the infection (p=0.00, p=0.04 and p=0.02 respectively). None of the family members were infected with the HDV. The main limitation of this study is that it is only a pilot study before establishing a global search strategy of HBsAg carriers. In conclusion, the HBV carriers search in families found a high prevalence of HBV and would help to identify chronic carriers that can be treated and contribute to a HBV elimination plan Key-words: Hepatitis B, intrafamiliar, carriers.

1398

IS IT EBOLA OR IS IT A VACCINE REACTION? EVALUATION OF SUSPECTED EBOLA CASES IN A VACCINE TRIAL DURING AN EBOLA EPIDEMIC: SIERRA LEONE TRIAL TO INTRODUCE A VACCINE AGAINST EBOLA (STRIVE)

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STRIVE is a phase 2/3 trial of rVSV-ZEBOV (Merck) candidate Ebola vaccine among healthcare and frontline response workers in Sierra Leone. The early symptoms of Ebola virus disease (EVD) typically include fever, headache, fatigue, and muscle/joint pain, similar to immediate vaccine reactions. Rapidly distinguishing EVD from vaccine reactions was a key component of STRIVE. Isolation and treatment were essential for true EVD, but referring participants with vaccine reactions to Ebola Holding Units (EHU) could increase the risk of Ebola exposure. In consultation with national response authorities, we developed a modified algorithm for management of EVD-like illness following vaccination. The national EVD case definition was temperature > 38.0oC and > 3 of the following 11 symptoms: headache, loss of appetite, fatigue, muscle/joint pain, diarrhea, unusual bleeding, difficulty breathing, nausea/vomiting, abdominal pain, difficulty swallowing, hiccups. The case definition was modified for participants vaccinated within the past 48 hours to require that one of the three symptoms be EVD-specific (diarrhea, unusual bleeding, difficulty breathing, nausea/vomiting, abdominal pain, difficulty swallowing, hiccups). Those vaccinated participants who did not meet the modified case definition could remain home and be followed for up to 24 hours if improving rather than referral to an EHU unless they reported direct, unprotected Ebola exposure or breach in the use of personal protective equipment in the prior 21 days. Participants referred to an EHU were tested by PCR for Ebola virus. To implement the modified algorithm, we trained EHU staff and Ebola hotline telephone operators to ask about participation in STRIVE and on post-vaccination symptoms and the modified algorithm. As of April 5, 2016, 48 STRIVE participants had been evaluated for suspect EVD; all were negative. The most common diagnosis was malaria, reported in approximately half. STRIVE was able to develop and implement a modified Ebola case definition to evaluate suspected EVD cases and prevent unnecessary admissions to EHUs during an Ebola outbreak.

1399

DEFINING A MULTIVALENT VACCINE AGAINST HEMORRHAGIC FEVER VIRUSES BASED ON INSECT CELL EXPRESSED RECOMBINANT SUBUNITS

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We have previously developed a preclinical Ebola vaccine candidate based on soluble recombinant Ebola virus (EBOV) glycoproteins (GP) and matrix proteins (VP24 and VP40) produced using the Drosophila S2 cell expression system combined with antigen-specific immunoaffinity chromatography purification. The immunogenicity and efficacy of this candidate has successfully been evaluated in mice, guinea pigs and macaque models. The lead candidate, which is protective in non-human primates, is undergoing additional pre-clinical development with a focus on defining correlates of protection. To broaden the efficacy profile of the core vaccine, GP subunits of Sudan virus (SUDV), Marburgvirus (MARV), and Lassa virus (LASV) have been expressed and purified using the same production platform. Having achieved a similar level of purity for the additional subunits, we started immunogenicity and preliminary efficacy testing in rodent models. As expected, GP subunits of the additional filoviruses show a similar

immunogenicity profile as EBOV GP with SUDV GP showing greater cross-reactivity with EBOV GP than MARV GP. With the use of clinically relevant adjuvants, potent antigen-specific IgG titers were observed after two or three immunizations of Swiss Wester (outbred) mice. Antigen balancing studies using the three filovirus GP proteins allowed the selection of candidate formulations that consistently achieve balanced humoral immunity to all three viruses. Current work focuses primarily on incorporation of the LASV GP to achieve a broadly effective formulation targeting all major hemorrhagic fever viruses with epidemic potential in sub-saharan Africa.

1400

DENGUE, ZIKA AND CHIKUNGUNYA CO-INFECTIONS AMONG ACUTE FEBRILE ILLNES PATIENTS IN SALVADOR, BRAZIL

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Chikungunya (CHIKV) and Zika viruses (ZIKV) have recently emerged in tropical countries of the western hemisphere, causing large epidemics in settings where dengue virus (DENV) transmission is endemic. Since all of these RNA arboviruses transmitted to humans by the same *Aedes spp*. mosquitoes, co-infections may occur regularly during outbreaks. However, as the manifestations in the initial stages of illness asued by thee viruses aresimilar and laboratory diagnosis are not widely available, detection of co-infections is uncommon. We investigated the frequency of DENV, CHIKV and ZIKV co-infections among acute febrile illness outpatients during a period of high arboviral transmission in Salvador, Brazil. RT-PCRs specific for DENV, CHIKV and ZIKV, as reported previously, were performed on acute-phase serum samples of 180 acute febrile illness patients who visited an Emergency Center in Salvador, Brazil between September 2014 and October 2015. An arboviral infection was detected in 46 (25.5%) of the 180 tested samples. Arboviral co-infections were detected in 5 samples (2.8% of the 180 tested samples and 10.9% of the 46 arboviral positive samples). DENV was detected in 16 (8.9%), CHIKV in 18 (10.0%), and ZIKV in 7 (3.9%) of the samples. DENV and CHIKV co-infection was detected in 4 (2.2%) samples, while DENV and ZIKV co-infection was detected in 1 (0.6%) sample. Among the samples that were solely positive for DENV, four were type 1, six were type 3, and five were type 4. All four samples that tested positive for DENV and CHIKV were DENV type 4, while the sample positive for DENV and ZIKV, wasDENV type 3. All five co-infected patients had fever, headache, retro-orbital pain, myalgia, arthralgia, prostration and rash, and recovered completely. In conclusion, our study indicates that arboviral co-infections are not a rare event in settings where DENV, CHIKV and ZIKV co-circulate. Apparently, co-infections do not change disease presentation and disease course, but further studies with larger number of patients are warranted to confirm this observation.

1401

KNOCK DOWN RESISTANCE (KDR) GENE IN *ANOPHELES COLUZZII* AND *AN. GAMBIAE* THAT SURVIVED THE DIAGNOSTIC CONCENTRATION OF PYRETHROIDS AND DDT IN TWO ECO-EPIDEMIOLOGICAL ZONES (GUINEA SAVANNAH AND COASTAL MANGROVE) OF NIGERIA

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The scale-up of long-lasting insecticidal nets (LLINs) has contributed significantly to the reduction of malaria morbidity and mortality in Nigeria. However, insecticide resistance remains a challenge. Here, we report susceptibility status to insecticides and the frequency of the kdr alleles in *Anopheles coluzzii* and *An. gambiae*. Susceptibility tests were carried out on *An. gambiae* s.l. mosquitoes from larval collections, with deltamethrin and DDT using standard WHO procedures. Additionally, adult mosquitoes were sampled inside houses using human-baited CDC Light Trap collections between May and September 2015 and were identified using morphological keys. These samples were subjected to polymerase chain reaction (PCR) assays for species identification and detection of *An. coluzzii* and *An. gambiae*. The kdr genotypes were determined both in *An. gambiae* s.l. collected indoors and those that survived insecticide exposure using allele-specific PCR tests. PCR results show that *An. coluzzii* and *An. gambiae* occurred in sympatry at both sites. However, *An. gambiae* predominated, representing 71.2% and 84.4% of the 500 *An. gambiae* s.l. tested in Nasarawa and Lagos, respectively. There was no detection of *An. coluzzii* + *An. gambiae* bands in any specimen suggesting the absence of hybrid state. The kdr diagnostic PCR showed the presence of the kdr-west mutation in 3.3% of samples collected indoors at Nasarawa, but in 27.0% of samples that survived deltamethrin exposure. In Lagos, kdr-west was present in 10.0% of samples collected indoors versus 28.8% in those that survived deltamethrin exposure. The difference between both sets of samples was statistically significant ($p<0.0001$) with a higher kdr frequency on samples that survived the diagnostic dosages than those collected indoors. More homozygotes were found among samples that had kdr, while none of *An. coluzzii* in both sites was positive for the kdr mutation. Overall, kdr frequencies were within the range recorded earlier in Southwestern Nigeria between 2002-2005. Further investigation is needed to learn the operational implication of the frequency of kdr genotype observed.

1402

INTERCEPTOR G2: A NOVEL LN FOR MALARIA CONTROL AND BEYOND

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The utility of bed nets to combat the scourge of malaria has been undeniably successful. However, a negative consequence of the

widespread use of long-lasting insecticidal nets or LNs, has been the accompaniment of selection which further exacerbates well-known resistance issues to neuro-toxic chemistries (like pyrethroids) for mosquitoes. The technical difficulties of utilizing other insecticidal active ingredients with alternative modes of action (beyond pyrethroids) has been a limiting factor for LN development, largely owed to mitigation needs for safety to sleepers (like pregnant mothers and children) that would use them and the physical-chemical nature of the insecticides' opportunity to be incorporated into LNs—most notably solubility of insecticides is challenging to overcome in order to insure they adhere to both the wash-resistance and efficacy performance profile recommended by WHOPES. Interceptor G2 achieves this difficult balance of needs. Interceptor G2 is a novel net unique among LNs, because it is truly the first net which includes two discrete adulticides, each with unique modes of action with both an excito-repellent component (alpha-cypermethrin) and a physiological insecticide (chlorfenapyr) that work in a concerted way to provide improved protection to LN users. This unique combination can provide significantly better efficacy to resistant mosquitoes. Field testing results from Benin, Burkina Faso and Tanzania have unequivocally demonstrated significantly higher efficacy to resistant mosquito strains (40-60% increased mortality to resistant strains). This net, developed by BASF through a partnership with IVCC holds great promise as a remarkable LN that can complement any area-wide efforts to protect malaria transmission unlike any other LN currently in the market, exploiting mosquito resistance mechanisms against themselves and affording improved protection to its users.

1403

WHO SUSCEPTIBILITY TEST VS. CDC BOTTLE BIOASSAY: COMPARISON OF THE CURRENT METHODOLOGIES FOR CONDUCTING INSECTICIDE RESISTANCE MONITORING

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Malaria incidence has dropped by 37% among populations at risk between 2000 and 2015 and death rates have decreased by 60% globally. Vector control interventions, namely insecticide treated nets and indoor residual spraying, have proven to be the largest contributors to the reduction in malaria incidence over the past 15 years. The extensive use of these insecticide based vector control techniques have selected for resistance in the *Anopheles* mosquitoes, the principal vectors of the malaria parasite. Numerous studies have demonstrated the presence of insecticide resistant mosquito populations throughout Sub-Saharan Africa. The World Health Organization (WHO) susceptibility test and the Centers for Disease Control and Prevention bottle assay are used to detect insecticide resistance within mosquito populations. To determine if the two methods are comparable, both assays were conducted on six laboratory reared insecticide resistant mosquito strains from three different *Anopheles* species: *An. gambiae* (RSP, ZAN/U and TORORO), *An. coluzzi* (AKDR, AKRON), and *An. arabiensis* (SENN). The six strains were tested against five compounds from three of the four classes of insecticides recommended by WHO: organochlorides (dieldrin and DDT), carbamates (bendiocarb) and pyrethroids (permethrin and deltamethrin). Results indicate definite differences in 24 hour mortality and knockdown rates between the two test methods with the CDC bottle assay often yielding significantly higher mortality. Significant variability, between methods as well as test reps, was seen across all mosquito strains when testing for organochloride (DDT) resistance. Both tests use the same standard for determining resistance: 98% to 100% mortality indicates susceptibility, 90% to 97% mortality suggests the possibility of resistance with additional tests needed, and <90% mortality confirms insecticide resistance. These test methods are important instruments for insecticide resistance surveillance systems and inconsistency between the two can lead to different insecticide resistance classifications and subsequent vector control program actions.

1404

CAN CHICKEN FEATHERS BE USED TO PRODUCE EFFECTIVE, RE-USABLE, DURABLE AND LOW-COST MOSQUITO NETS?

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Treated mosquito nets have dramatically reduced indoor malaria transmission. However, they are not readily accessible and affordable. Among challenges of existing nets include easily torn-out and fail to inhibit blood feeding after few years of use. Here we study possibilities of using chicken feathers, a waste by-product, to produce low cost, durable, reusable, effective, and community affordable mosquito nets that adheres to WHO standards. Initial stages prior to making the nets involved laboratory tests similar to WHO cone test (i.e., regeneration time, wash-resistance, and efficacy) against pure feathers, and on fabrics material made from chicken feathers. The tests were performed on *Aedes aegypti* using permethrin. The preliminary results indicate that pure feathers and made fabric material can absorb and retain insecticide. Pure feathers had 100% knockdown and mortality effect, and made fabric had 80-100% knockdown and mosquito mortality. These results were read after 3 consecutive washes (i.e., 48, 72, and 96 hrs). The promising findings from initial stages indicate that there are possibilities of using chicken feathers to potentially produce effective, re-usable, durable, and affordable mosquito nets. Such nets will be subjected to semi-field WHO cone test using malaria vectors with different insecticide before comparing them against commercialized mosquito nets. Although initial thought of the study is based on mosquito carrying malaria parasite, the same net can also be effective against mosquitoes carrying Zika virus.

1405

TRANSCRIPTOME ANALYSIS OF GENES ASSOCIATED WITH DELTAMETHRIN RESISTANCE IN *ANOPHELES ALBIMANUS*

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Malaria remains one of the most important and debilitating diseases in the tropical world. One of the greatest emerging threats to malaria prevention and control is the development of insecticide resistance in the mosquitoes that transmit malaria. In the Americas, as malaria programs begin to shift their focus to regional malaria elimination, insecticide-based vector control strategies will intensify. Decades of unmanaged insecticide use and routine exposure to agrochemicals have left many populations of malaria vectors in the Americas resistant to multiple classes of insecticides, including pyrethroids, which are most cost-effective class of public health insecticides currently available. Comparatively little is known about the molecular basis of this resistance. Diagnostic tools are urgently needed to understand how malaria vector control interventions could be compromised by insecticide resistance. Applying a genomics approach utilizing advanced molecular detection tools, this project aims to comprehensively characterize mechanisms of deltamethrin (a pyrethroid insecticide) resistance in one of the most important malaria vectors in the Americas, *Anopheles albimanus*. *An. albimanus* were collected from Tumbes, Peru in 2015 and phenotyped as deltamethrin-resistant or susceptible using the CDC bottle bioassay. Whole non-ribosomal RNA from field-collected resistant, field-collected non-exposed, and a susceptible laboratory strain of *An. albimanus* are being analyzed using Illumina-Hiseq sequencing for comparison of gene expression profiles. Preliminary data will be presented, as well as a discussion of how these data will be used to develop mechanism-specific assays.

1406

INSECTICIDE RESISTANCE PROFILE OF *ANOPHELES GAMBIAE SENSU LATO* IN AREAS WITH AND WITHOUT INDOOR RESIDUAL SPRAYING IN MALI, WEST AFRICA

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In Mali, long lasting insecticide-treated nets (LLINs) are at the frontline of malaria vector control tools. Since 2008, US President Malaria Initiative (PMI) is supporting the implementation of an Indoor Residual Spraying (IRS) in three districts (Koulikoro, Baroueli, and Bla) in addition of the LLINs. Insecticide used for IRS has been changed from pyrethroid to carbamate and then to organophosphate because of the development of insecticide resistance by malaria vectors. The objective of this study is to determine the insecticide resistance profile of *Anopheles gambiae* s.l. in areas where LLINs together with IRS (Koulikoro district) are being implemented compared to areas with LLINs alone (Banamba & Kati districts). WHO bioassay tests were performed on F0 and/or F1 progeny of *An. gambiae* s.l. from larvae and/or from female adults, respectively, to assess their phenotypic resistance. The Taqman method was used to determine the different resistance mechanisms underlying the phenotypic resistance. A very strong phenotypic resistance was observed in all investigated localities in both areas. The 24 mortality rates were 6%, 29%, 29% and 30% (N=100) respectively in Koula, Karadie (in IRS area), and in NGalamadibi and Dangassa (non IRS area). Both West (L1014F) and East (1014S) kdr resistance mechanisms were observed in all localities. The frequency of the West form (43.54%; N = 182) was 2.5-fold higher than that of the East form (17.22%; N = 206) in LLINs+IRS areas compared to LLINs areas. Glutathione S transferase genes "GSTe2" (L119) and the Ace gene (N645I) were observed only in IRS+LLINs areas. This study showed that metabolic resistance mechanisms were encountered in area where IRS is associated to LLINs and absent in nearby area where LLINs are being implemented.

1407

EVALUATING HETEROGENEITY IN INSECTICIDE SUSCEPTIBILITY FOR IMPROVED RESISTANCE MANAGEMENT STRATEGIES TO AID MALARIA ELIMINATION

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Southern Mozambique is currently designing and piloting activities to eliminate malaria by 2020. Insecticidal vector control measures are an important component of the elimination strategy. However, insecticide resistance in malaria vectors is a critical threat to malaria control and elimination programs. As such the longitudinal monitoring of insecticide susceptibility is crucial to select appropriate vector control tools, but also to inform insecticide resistance management plans and generate the essential biological data needed for mathematical modeling aimed at designing novel resistance management plans. Here we show detailed insecticide resistance data for the major malaria vectors (*Anopheles funestus* and *An. arabiensis*) in Manhiça and Magude districts, obtained by WHO tube and CDC bottle bioassays. We studied spatial heterogeneity in insecticide susceptibility on different spatial scales at district, village and neighborhood level, aimed at identifying the barriers to the spread of resistance that will affect insecticide resistance management strategies.

1408

INSECTICIDE RESISTANCE IN *ANOPHELES GAMBIAE* AND *AN. FUNESTUS* IN TWO RURAL SITES IN TANZANIA

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Over the last decade, considerable reductions in global malaria burden have been achieved by scaling-up key vector control strategies across endemic areas. However, long-term effectiveness of both long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) is currently under threat from widespread emergence of insecticide resistance, especially resistance to pyrethroids. To characterize levels of insecticide resistance across Tanzania, CDC bottle bioassays were undertaken in two rural sites: Muleba (Northwest, Kagera region) and Muheza (Northeast, Tanga region). Insecticides tested were pyrethroids permethrin and alphacypermethrin. *Anopheles gambiae* and *An. funestus* were collected through resting indoor catches (Muleba) and larval collections (Muheza). Kisumu (susceptible *An. gambiae* strain) were used as a comparison where available. Resistance frequency was tested by exposing mosquitoes to the diagnostic dose of each insecticide and resistance intensity by using a range of doses. In Muheza, mortality in *An. gambiae* after 30 minutes exposure was 73% for 21.5µg/ml of permethrin and 70% for 12.5µg/ml of alphacypermethrin. All insecticides produced 100% mortality in Kisumu. In Muleba, only 42% of *An. gambiae* died after exposure to permethrin (21.5µg/ml), as well as 430µg/ml (20x the diagnostic dose) producing only 79% mortality and 645µg/ml (30x the diagnostic dose) 94% mortality. 56% of An. *funestus* died after exposure to 21.5µg/ml of permethrin. These results suggest that pyrethroid resistance is present in *An. gambiae* and An. *funestus* in both locations, and at particularly high levels in Muleba. Research into pyrethroid resistance, as well as resistance to other insecticide classes, is ongoing in Muheza. This research is vital in determining the appropriate vector control strategies to continue the decrease in malaria prevalence in these locations.

1409

KDR MUTATIONS CONFER FITNESS COST AND COMPETITIVE DISADVANTAGE IN FIELD POPULATIONS OF *AEDES AEGYPTI*

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Vector control strategies for *Aedes aegypti* are being threatened by the evolution of insecticide resistance. Point mutations in the *para*-orthologous sodium channel gene confer resistance to pyrethroid insecticides, named *kdr* for "knock-down resistance." While these mutations increase survival in the presence of insecticide, they also carry a fitness cost which could allow populations to recover susceptibility in the absence of insecticides. Additionally, resistant individuals could be inferior competitors to susceptible ones when subjected to density dependent competition. To investigate the fitness cost and competitive ability of *kdr* individuals, we conducted experiments with two field populations of *Ae aegypti:* a susceptible population with 1% *kdr* frequency of I1016 and zero C1534 mutations, and a resistant population with 100% frequency of C1534 and 73% frequency of I1016. First instar larvae from each population were placed in separate 1L buckets in two density treatments: 50 larvae or 500 larvae. A third population consisted of a 50/50 mix of individuals from each original population to test competitive ability. After all mosquitoes emerged, a subset of adults were bloodfed and eggs per female were enumerated to estimate fecundity. All populations laid fewer eggs per female in the high density treatment than the low (ANOVA,

F=5.6, p=0.031), though both the susceptible and mixture populations produced 4.3 times more eggs per female than the resistant population after controlling for density (GLM, F=9.68, p=0.0008), indicating a significant fitness cost to the *kdr* mutations. CDC bottle bioassays were also conducted on each treatment with the diagnostic dose of permethrin. The resistant population performed significantly worse in the high density treatment than the low, with a 93% knock-down rate at the diagnostic time in the high density compared to 52% in the low density (t-test, t=-2.6, p=0.05). These results suggest that the resistance phenotype can be altered by ecological interactions in the larval stage. Future control strategies could exploit the fitness and energetic cost of *kdr* to regain susceptibility into populations.

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DYNAMIC OF MALARIA VECTORS SUSCEPTIBILITY TO PYRETHROIDS AND MECHANISMS OF RESISTANCE IN SENEGAL

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Malaria vector control in Senegal rests on long-lasting insectidal nets (LLINs) and indoor residual spraying (IRS). Since 2007, IRS was implemented in selected districts, with pyrethroids until 2010, and then with carbamates. LLINs are distributed nationwide, with a universal coverage strategy since 2010. To monitor vector sensitivity to insecticides, sentinel sites were established to reflect the epidemiological strata of Senegal and the use of IRS. Annual monitoring was carried out at sentinel sites from 2008-2013 using World Health Organization (WHO) insecticide-impregnated papers. Tests were performed on an F1 generation aged 2 to 5 days, collected from wild *Anopheles gambiae s.l.*, with 25 female subjects in each of four tests, and a control of 25 females. Tests were interpreted according to the following criteria: mortality of 98-100% was considered sensitive, 80-97% considered suspected resistance, and less than 80% resistant. Sensitivity to the pyrethroids, deltamethrin and permethrin was assessed in 2 IRS districts (Velingara and Nioro) and 2 control district (non IRS). " The vector susceptibility to pyrethroids increased in IRS districts after the shift to carbamates (2011), even though universal coverage of LLINs started the same year (2010-11). " The same tendency is not observed in non IRS districts. " Increase in resistance to pyrethroids not convincingly seen with introduction of universal coverage of LLINs. " The vectors are susceptible to carbamates and organophophates in both IRS and non-IRS districts, but resistant to organochlorines in both IRS and non-IRS districts. The presence of Kdr-east is preliminarily detected in Nioro and this allelic frequency seems to show a fixation of this gene in field Anophelines. Additional analyses are underway to search for other mechanisms of resistance, other than the mutation of the target involving the Kdr gene. Preliminary evidence indicated the presence of the metabolic resistance among vector populations from several localities in the country.

1411

SPATIO-TEMPORAL EVOLUTION OF RESISTANCE TO DELTAMETHRIN AND KDR MUTATIONS IN *AEDES AEGYPTI* POPULATIONS IN FRENCH GUIANA: A WORRYING SITUATION FOR VECTOR CONTROL

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Aedes aegypti is vector of dengue, chikungunya and zika viruses in urban area of French Guiana, a French territory in South America. Deltamethrin remains the sole insecticide molecules authorized for adult control in the European Union to which French Guiana belongs. Since 2009, resistance to deltamethrin has been monitored in several populations of *Ae. aegypti* from French Guiana, by using WHO test kit at a diagnostic dose of 0.06%. In complement, mutations located at the position 1016 and 1534 of the sodium voltage-gated channel gene were monitored in some of these populations by Taqman Allelic Discrimination Assays. These mutations were already linked to pyrethroid resistance in *Ae. aegypti* populations from Latin America. A high resistance level was observed even before 2010, year from which deltamethrin has been outdoor sprayed. Our study also demonstrated a spatial and temporal heterogeneity of both mortalities at the diagnostic dose (from 1% to 92%) and resistant allele frequencies (from 14 to 98% of I1016 and from to 31 to 100% of C1534). Those frequencies have increased from 2009 to 2015. These results highlight a worrying situation for vector control efficacy and public health concern in French Guiana with no other insecticide yet authorized. Alternative control strategies will be discussed regarding these results.

1412

ONE YEAR OF COMMUNITY LED LARVICIDING :BIOKO ISLAND

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Background Larviciding has historically recorded success in vector control, known to be effective in urban setting where breeding sites are generally and easily assessable. This study is aimed accessing acceptability and implementation of a community led larviciding in a rural setting. Methods Community Agents (ACPs) were identified and recruited in 13 communities with the help of the community leaders; trained and provided materials. At baseline , the entomology team and the ACP identified and characterized existing habitats; type of habitat, existence of larvae and the stages of larvae. Weekly, the ACPs were expected to visit and treat all habitats that the owners consented and identify and treat new ones. The leader of the ACPs kept a register of attendance. The entomology team visits the community on the same day of the week but at a later time to monitor the activities of the ACP and recorded; if the each habitat has been treated, habitats are categorized as being old or new, the stages of larvae and updated the ACP's attendances register. Periodically, the ACPs were given a feedback on their activities at a community meeting. Result: At baseline there were 1100 habitats in the 13 communities, about 64.0% of the habitats were in only 2 communities, there were 92 ACPs. About 83.2% of the habitats were household water containers, 4.6% car tracks, and the drainage system being 4.5%. In all 2.9% of the habitats were *Anopheles* positive and 38.5% were positive for another culicine mosquitoes. Weekly an average of 1376 habitats were to be treated, 25(1.9%) were new, 82(6.0%) were not treated because the owners rejected treatment. The weekly average of habitats treated by ACPs was 76.0 %(std 7.8%), the average attendances to treatment activities is 82.0%(std 9.2%) Conclusion: Larviciding requires 100% treatment of all known habitats to be successful. The degree of refusal to accept larviciding

and the non-attainment of the 100% coverage by the ACPs suggest community led larviciding needs a closer look at regards community entry and how the ACPs were chosen.

1413

INDOOR RESIDUAL SPRAYING FOR MALARIA CONTROL USING BENDIOCARB REDUCES *KDR* L1014S HOMOZYGOTE FREQUENCY IN *ANOPHELES GAMBIAE* S.S.

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Resistance of malaria vectors against pyrethroid insecticides has been attributed to selection pressure from long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and agricultural chemicals. The use of different classes of insecticides in combination or by rotation has been recommended for resistance management. The aim of this study was to evaluate the role of using carbamate insecticides for IRS in the management of resistance against pyrethroids used in LLINs. *Anopheles gambiae* s.s. were collected from 33 different sites in nine districts of Uganda, three of which were under bendiocarb spraying. The *kdr* L1014S homozygote (RR) frequency (*kdr* homozygosity) was used as the outcome variable to test the effects of various factors using a logistic regression model. Spray status with bendiocarb, annual rainfall, altitude, collection type (larvae or adults), long-term LLIN use estimated from old nets found hanging, LLINs distributed in the previous five years, household use of agricultural pesticides, and intensity of malaria transmission (prevalence in children 2-9 years old) were entered as explanatory variables. Spray status, collection type and annual rainfall had statistically significant effects. *A. gambiae* s.s. collected from areas sprayed with bendiocarb had significantly lower *kdr* homozygosity than those collected from non-sprayed areas. Mosquitoes collected as adults from indoor collections had significantly higher frequency of *kdr* homozygotes than mosquitoes collected as larvae, possibly indicating selective sampling of resistant adults following exposure to insecticides inside houses. Sites with high rainfall had significantly lower *kdr* homozygosity. The results indicate that bendiocarb spraying may potentially increase susceptibility of *A. gambiae* s.s. to pyrethroids by reducing *kdr* resistant genotypes. Although bendiocarb spraying did not result in elimination of the *kdr* resistant genotypes, the analysis indicated that IRS with carbamates, or possibly also organophosphates, could have a role in pyrethroid resistance management.

1414

ENGINEERED *ANOPHELES GAMBIAE* IMMUNITY TO *PLASMODIUM* INFECTION

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Impairing sporogonic development of *Plasmodium* using the transgenic manipulation of its mosquito vector has been achieved in different malaria-transmitting species. We have previously generated immune-enhanced *Anopheles stephensi* mosquitoes with greater resistance to *Plasmodium* and microbial infection through transgenic over-expression of the IMD pathway-controlled Rel2 transcription factor, as reported previously. Despite the relevance of *Anopheles gambiae* (A. *gambiae*) as the major human malaria vector in sub-Saharan Africa, it has not been extensively used as a vector model for transgenic research because of being technically more cumbersome to transform. In the present study, we have successfully developed a genetically modified immune-enhanced A. *gambiae* line, by over-expression of the FBN9 gene, hence overcoming previous technical challenges. Engineered anti-*Plasmodium* activity of the IMD pathway has been further explored by investigating the potential of the Rel2-regulated anti-*Plasmodium* immune factor FBN9 (fibrinogen immunolectin 9) to confer resistance to *Plasmodium*. We used the fat body-specific blood meal-inducible Vitellogenin 1 promoter to drive transgene expression of this anti-*Plasmodium* factor. The promoter, FBN9 gene and the Trypsin 1 terminator were ligated into an entry vector containing the DsRed marker. Newly laid eggs from female A. *gambiae* mosquitoes (G3 strain) were injected and larvae screened for transient fluorescence. Transient mosquitoes were outcrossed with wildtype ones and, following blood feeding, the offspring was screened for transgenics. FBN9-transgenic mosquitoes showed increased resistance to *Plasmodium*, by reduction of both infection prevalence and intensity at the oocyst stage. The temporal expression pattern of the recombinant FBN9, the antibacterial response of the immune-enhanced transgenic mosquitoes and the impact of this genetic modification on mosquito fitness have been analysed.

1415

DISPERSAL AND THE SPREAD OF A DENGUE-SUPPRESSING BACTERIUM IN THE DENGUE VECTOR *AEDES AEGYPTI*

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Wolbachia is a promising tool for arbovirus control, with its potential to suppress virus transmission and to establish and spread through wild mosquito populations under the right conditions. *Aedes aegypti* infected with the *w*Mel strain of *Wolbachia* were released into two suburbs of Cairns, Australia. At each site, *Wolbachia* spread was spatially heterogeneous, and slower than initially expected. Also, the invasion appeared to stall at the boundary of a major road, suggesting this to be a strong barrier to mosquito dispersal. Conversely, a rapid influx of uninfected mosquitoes to the centre of one of the sites was observed suggesting long distance dispersal of *Ae. aegypti* in Cairns. To test this further, we investigated spatial genetic structure among 161 field-caught *Ae. aegypti* from the same area using ddRADseq. We observed little genetic structuring across the range of our study site (4 km^2) and found a weak barrier effect of roads. These findings support our hypothesis that long-range dispersal is common in *Ae. aegypti* in Cairns. A highly leptokurtic distribution of dispersal distances can lead to slower *Wolbachia* spread and the potential reinvasion of infected regions in seasonally dynamic populations, these findings thus inform future releases of *Wolbachia.*

1416

IDENTIFICATION OF MIDGUT ACTIVE CIS-REGULATORY SEQUENCES TO EVALUATE THE IMPACT OF NON-CODING VARIATION ON *AEDES AEGYPTI* SUSCEPTIBILITY TO DENGUE VIRUS INFECTION

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The basal level of expression of immunity genes is correlated with *Aedes aegypti* strain susceptibility to Dengue virus, as reported previously. The basal level of expression is determined in large part by the set of cis-regulatory sequences that are active in midgut cells. A portion of the variation in the basal level of gene expression in the midgut is potentialy related to variations in cis-regulatory sequences. Therefore, in order to have a genomic template cis-regulatory sites to analyse the impact of non-coding variations on strain susceptibility, we set out to identify and characterize cis-regulatory sequences active in the non-stimulated midgut of *Ae. aegypti*. Since open chromatin is a hallmark of cis-regulatory function we carried out open chromatin profiling on pools of disected midguts from 3 to 5 day old female mosquitoes. Genomic libraries were constructed with DNA samples enriched for cis-regulatory fragments.

These genomic libraries were then sequenced by NGS in an Illumina GAx II instrument. More than ten million non-redundant, uniquely mapping sequence tags were used to map enriched peaks corresponding to cis-regulatory sequences. Analysis of these sequence tags by MACS and DFilter identified more than 30 thousand cis-regulatory sequences. Characterization of these cis-regulatory sequences allowed identification of motifs and cis-regulatory modules (CRMs) for the binding of a diversity of transcription factors. This set of cis-regulatory sequences, motifs and CRMs will be useful to assess the potential impact of non-coding variation on *Aedes aegypti* susceptibility to Dengue virus infection.

1417

MOLECULAR CHARACTERIZATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY SPECIFIC VARIANTS IN AMHARA REGION, ETHIOPIA

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Glucose 6-phosphate dehydrogenase deficiency (G6PDd) is an X-linked hereditary genetic defect, affects an estimated 400 million people worldwide. Severe clinical manifestation associated with G6PDd (e.g., chronic hemolytic anemia) depends on the type of G6PD molecular variants and exposure to hemolytic triggers (e.g., antimalarial like Primaquine). However, scarce studies on G6PDd renders the use of Primaquine for effective therapeutic treatment of malaria. This study was undertaken to determine the availability and characterize selected molecular variants of G6PDd specific genes among selected populations in malaria endemic area of Ethiopia. Using cross sectional study design a total of 156 dried blood samples were randomly selected from 360 stored samples of national malaria indicator survey of 2011 starting from July 30/2014 to January 30/2015. Polymerase chain reaction and restricted fragment length polymorphism technique was applied to characterize G6PDd variants as G6PD*A, G6PD*A- and/or G6PD*Mediterranean. Binary logistic regression was applied to see association (P<0.05 is significant) among different parameters. Of 156 studied dried blood spot samples, 10(6.4%) had G6PD genotype available. G6PD*A (100%) was the only genotype characterized, while neither G6PD*A- nor G6PD*Mediterranean genotypes were detected. There was no statistical significant difference between G6PDd and other socio demographic and risk related variables (P>0.05). In conclusion, G6PD*A variant was the only G6PDd genotype detected in this study. G6PD*A variant has almost (90%) the same enzymatic activities with the wild type. Therefore; this result supports the safe use of primaquine, especially the single low dose for transmission interruption of *Plasmodium falciparum* gametocyte and radical cure of *P. vivax*, as a part of malaria elimination toolkit, among selected populations in malaria endemic areas of Amhara region.

1418

UNIDIRECTIONAL HYBRIDIZATION AND REPRODUCTIVE BARRIERS BETWEEN TWO BIOTYPES OF *CULEX PIPIENS* COMPLEX

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Understanding the processes of reproductive behavior in mosquitos is crucial for improving mating competitiveness and mating specificity for sterile insect release program. *Culex pipiens* form pipiens and form molestus, two biotypes for *Cx. pipiens* complex, are vectors for West Nile Virus, St. Louis encephalitis virus, and lymphatic filariases. Hybridization of these biotypes is shown to occur in nature, even though f. pipiens mate above ground in larges spaces (eurygamy) and f. molestus preferentially in small spaces (stenogamy) like subways and sewage tunnels. The hybridization between two biotypes may allow gene flow of biotype-specific characteristics that are crucial in the disease transmission cycle. In the present study, we examined the mating behaviors, insemination rates, fecundity and fertility in F1 hybrids between *Culex pipiens* form pipiens and f. molestus in stenogamy conditions (cage 27.5cm x 17cm x 20cm). The F1 hybrid crosses along with parent backcrosses were also accessed for mating success. Despite the considerably high insemination rates from hybrid males to females and likewise in backcross lines to parent females, the fertility and fecundity rates from the respective females were varied among different crosses. This observation could suggest the asymmetric allele introgression in the hybrid zone. We also document a failure of heterospecific males to produce fertile eggs in f. pipiens females, which may be due to gametic incompatibilities and may serve as an additional barrier to gene exchange.

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SMALL INSERTION AND DELETION MUTATIONS IN *ANOPHELES COLUZZII* AND *AN. GAMBIAE*

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Whole genome resequencing experiments now routinely leverage large data sets to explore genomic variation in organisms from diverse taxa, including arthropod vectors of disease. In these vectors, resequencing has the potential to detect cryptic populations, uncover signals of insecticide resistance, and monitor the progress of control campaigns, along with other contributions to disease eradication. However, these experiments often focus wholly on single nucleotide polymorphisms (SNPs), excluding small insertions and deletions (indels) as well as large structural variants. While current short-read technology makes examination of the latter fraught with technical artifacts, routinely including indels in the analysis of resequencing data is a way to increase the power of these experiments as well as detect patterns that cannot be uncovered using SNPs alone. We reanalyzed previously published short read data from 38 samples of *Anopheles gambiae* and *An. coluzzii*, focusing on variants longer than one base pair. We find that similar numbers of base pairs are involved in indel variants as in SNPs in this data set, suggesting that indels are indeed an important category of variation in these malaria vectors. We also find that pipelines commonly used for the detection and quality control of SNPs can easily be adapted for indels, and that indels analyzed this way show similar population genomic trends to SNPs, suggesting their reliability. Finally, we examine patterns of frameshifts in these malaria vectors, identifying genes whose expression may be radically altered in a way SNP data cannot easily detect.

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MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF *ANOPHELES MELAS* POPULATIONS IN ENDEMIC AND NON-ENDEMIC LYMPHATIC FILARIASIS COMMUNITIES IN COASTAL GHANA

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Anopheles melas, known to be an efficient vector of lymphatic filariasis occurs along the coastal part of Ghana, especially in estuarine areas. This study was aimed at seeking explanation to why there is different patterns of lymphatic filariasis distribution along coastal Ghana. The hypothesis was that the eastern *An. melas* populations could be different from those occurring to the west of Accra at both morphological and molecular levels. Two sites representing communities to the east of Accra in the Ga

Adangme District and two communities in the Ahanta West District were selected for the study. The mosquitoes were molecularly identified as *An. melas*. Deoxyribonucleic acid (DNA) of each identified *An. melas* was then used as the template for COI analysis. Polymerase chain reaction products obtained were purified, sequenced and analyzed using BioEdit and MEGA V6 software for construction of phylogenetic tree.In-silico restriction enzyme digest was done to determine restriction site differences between the two *An. melas* populations and Similarity index determined using Sørensen's formula. The study found no significant differences between the sequences of the two *An. melas* populations (Z-test of neutrality = 0.33). Results of the phylogenetic analysis though not significant, revealed a geographic relationship only between two eastern populations clustering together, with one population branching off on a different node. Analysis of the in-silico showed eight mutational differences with QS value of 0.99. The restriction site analysis however revealed 16 unique site differences between them. The mean numbers (n, median and range) of cibarial teeth were 14.54 (n = 13, 15, 11 = 18) and 14.1 (n=13, 14, 11-19) for western (Azizanya) and eastern (Asemko) respectively, which were not statistically different (t=1.53, P = 0.14). In conclusion the two *An. melas* populations could be said to be similar in all aspects, however restriction enzyme differences could be potential markers for distinguishing the two populations.

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POPULATION STRUCTURE OF *ANOPHELES FUNESTUS* IN SOUTHERN AFRICA

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Malaria control and eradication faces many challenges; among these is *Anopheles funestus*, one of three major malaria vectors in sub-Saharan Africa. *An. funestus* poses a significant threat because of its expansive distribution and high rates of insecticide resistance. However, relatively little is known about the population structure and dynamics of *An. funestus* compared to other major malaria vectors such as *An. gambiae* and *An. arabiensis*. In this study, individual samples (N=44) from geographically distant sites in Zambia, Democratic Republic of the Congo, and Tanzania were subject to whole genome sequencing for determining the degree of population structure of *An. funestus* in Southern Africa, and for the development of genome-wide markers suitable for population genetic studies at the spatial scale of this region. A reliable set of single nucleotide polymorphism markers will allow for high-throughput and cost-effective population genomic analyses at the spatial scale required by this study. Preliminary assessment suggests that more gene flow than expected exists between populations from Zambia and DRC, however a more detailed analysis is required. Robust estimate of gene flow between populations, especially related to insecticide resistance genes, could be used to assess efficacy of vector control. Moreover, association of whole-genome-based genetic clusters in relation to our discovery of sympatric *An. funestus* mitochondrial Clade I and Clade II in northern Zambia could illuminate if mitochondrial clades are relevant to mating structure within the *An. funestus* complex. Additional structure within the complex, if related to ecological or behavioral traits, would have implications for vector control strategies.

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GENERATION OF *AEDES* GENE KNOCKOUTS TO CHARACTERIZE LIGHT-DRIVEN PHOTORECEPTOR RESPONSES

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Daily shedding and rebuilding of the photoreceptor's photosensitive rhabdomeric membranes is common in invertebrate species. *Aedes aegypti* shows a robust response and loses all Aaop1 rhodopsin from the rhabdomeric membranes during the shedding process at dawn. We seek to characterize these cellular processes and determine how modulation of visual capabilities during this daily cycle impacts mosquito vision and behavioral responses. The shedding process at dawn is a light-triggered event. The rhodopsin is moved to cytoplasmic vesicles and degraded during the daytime. However, the rhodopsin is capable of being rapidly moved back to the rhabdomeric membranes if dark conditions are restored prior to degradation. During the daytime period, vesicles containing newly synthesized Aaop1 rhodopsin accumulate within the cytoplasm. At dusk, these vesicles rapidly deposit newly synthesized Aaop1 into the rhabdomere. These results show that light is a negative regulator of rhodopsin maturation and document the extensive management of rhodopsin content during the daily light-dark cycle in *Aedes* mosquitoes. We propose that these events give the mosquito exceptional visual capabilities in the low light environments of dawn and dusk without triggering light-induced damage to photoreceptors upon exposure to brighter daylight. We are testing this proposal by creating germ-line mutations in the Aaop1 rhodopsin gene using CRISPR-CAS9 technology. We also will create mutations in the two arrestin genes coding for adapter proteins responsible for initiation of the membrane shedding process. These three mutant strains will be examined for rhodopsin movement, retinal degeneration, and behavioral responses to determine the importance of proper rhodopsin management to *Aedes* fitness.

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USE OF THE OXFORD NANOPORE MINION MKI FOR SIMULTANEOUS VECTOR AND PATHOGEN IDENTIFICATION

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Vector-borne disease surveillance in remote locations is difficult at best. The vast majority of sequencing and antibody detection equipment on the market is bulky, delicate, and expensive. The MinION is a pocket-sized DNA sequencer that has previously been used in the field to track the Ebola outbreak in West Africa. The MinION has the potential to greatly expand vector surveillance capability in the US military. To this end, the Navy Entomology Center of Excellence, the USDA Center for Medical, Agricultural, and Veterinary Entomology, and Naval Medical Research Unit 3 (Cairo) are developing a protocol for simultaneous vector and pathogen identification using the MinION. Whole DNA is extracted from vectors using standard kits and prepared for sequencing using the simplest available protocol. The prepared libraries are sequenced using the MinION and locally BLASTed against a database of vector sequences for identification. A test run consisting of 5 mosquito species combined into a single library shows that a sufficient number of high quality 2D reads are produced to allow genus-level identifications within just a few hours. Next steps include expanding the reference database to include pathogen sequences, protocol testing on laboratory-infected mosquitoes, and further simplification of the protocol for field expediency.

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HUMAN-DISEASE CAUSING ARBOVIRUS PREVALENCE IN KENYAN MOSQUITOES

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Arboviruses comprise some of the most important emerging pathogens due to their geographic spread and increasing impact on vulnerable human populations. Despite this significant global health burden, the transmission, epidemiology, and incidence of arboviruses remains poorly defined, particularly in sub-Saharan Africa. In Kenya, the continued population growth and associated urbanization are conducive to proliferation of mosquito vectors and arboviral transmission; thus the characterization of arboviral circulation in this region is imperative to better inform human risk assessments and vector control practices. We used a variety of trap types and capture methods to collect *Aedes* and *Anopheles* species mosquitoes, at varying stages of the life cycle and during different seasons, at four sites in Kenya: Msambweni and Ukunda on the coast, and Chulaimbo and Kisumu in the west. Mosquitoes were then sorted by species, sex, trap type and date of capture, and grouped into 391 pools of ~25 individuals. Tissue was mechanically lysed and total RNA was extracted. Using a multiplex real-time reverse transcriptase PCR assay, mosquitoes were tested for dengue (DENV) and chikungunya (CHIKV) viruses, as well as for the five *Plasmodium* species known to cause human disease. CHIKV was detected in 14 of 290 (4.8%) of *Aedes* spp. pools. Of these, 3 were from the western sites, caught between March and May 2014, and 11 were from the coastal sites caught between July and December 2014 in ovitraps, human-landing catches and BG sentinel traps. Interestingly, 8 of these CHIKV positive pools were male mosquitoes bred in the laboratory from ovi- and larval traps, suggesting transovarial transmission of these viruses. DENV was detected in 1 pool (0.3%) from the coastal sites from September 2014. Of the 101 *Anopheles* pools tested for the five *Plasmodium* spp., 1 pool (1.2%) tested positive for *falciparum falciparum*. These data suggest a considerable prevalence of CHIKV in Kenyan mosquitoes, and that viral distribution varies both geographically and temporally. These data contribute to arboviral surveillance in Kenya, and suggest that the prevalence of CHIKV is underestimated.

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ECOCLIMATIC DRIVERS OF SPATIO-TEMPORAL HOT SPOTS OF *AEDES ALBOPICTUS* ABUNDANCE IN A SOUTH EUROPEAN URBAN AREA

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The stable colonization of several south European urban areas by *Aedes albopictus* represents an increasing public health threat due to the species competence in transmitting Dengue, Chikungunya and Zika arboviruses, whose expanding worldwide distribution is increasing the risk of an infected traveller to reach Europe. In fact, a Chikungunya outbreak has already occurred in northern Italy in 2007 and cases of autochthonous Dengue transmission have been recently reported from France and Croatia. Despite in the absence of vaccines the only way to prevent the risk of outbreaks of these diseases in Europe is mosquito control, this is rarely efficiently carried out by public administrations due to lack of appropriate resources to cover the large areas colonized by the species. It has been proposed that a more cost-effective method to prevent arbovirus outbreaks could be the focal treatment of hot-spot of highest mosquito densities. The aim of this work was to identify eco-climatic drivers of higher *Ae. albopictus* abundance on the basis of data from seasonal-round monitoring carried out in 2012-2013 across and beyond the urban area of Rome. A fine scale (300 m radius) spatio-temporal dataset was built within each sampling site and exploited to analyse the effect of climatic (Land Surface Temperature, Daily Rainfall, Growing Degree Days), environmental (Land Cover as retrieved from digital multispectral aerial imagery) and demographic (human population density) variables on *Ae. albopictus* spatial abundance and temporal dynamics. Generalized additive mixed models highlighted a strong positive relationship between mosquito abundance and anthropic surfaces and population density and identified climatic drivers of the seasonal population dynamics. These results provide useful indications to prioritize public mosquito control measures in temperate urban areas in space and time for a more feasible and cost-efficient prevention of the risk arbovirus transmission in Europe.

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OUTDOOR EARLY BITING BEHAVIOR AND INSECTICIDE RESISTANCE IN *ANOPHELES ARABIENSIS* MIGHT CHALLENGE MALARIA ELIMINATION IN SOUTHERN PROVINCE OF ZAMBIA

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In Zambia, long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) are the principal malaria vector control interventions. The use of these interventions depends on vector susceptibility to insecticides and indoor biting and resting behavior. However, there is limited information on the behavior of malaria vectors and their susceptibility to insecticides in Southern Province, an area targeted for malaria elimination. This study assessed vector behavior and susceptibility to insecticides commonly used in malaria vector control strategies. Indoor host seeking mosquitoes were collected during April and May 2015 to determine mosquito biting behavior. Species identification by PCR and insecticide susceptibility tests were conducted on 0.05% deltamethrin, 0.1% bendiocarb, 4% DDT and 0.25% pirimiphos-methyl following the WHO standard protocol. Metabolic resistance were determined in populations of *An. gambiae* s.l and *An. funestus* s.l. by using a synergist piperonyl butoxide (PBO). A total of 5,507 adult *Anopheles* mosquitoes were collected from April to May 2015. *An. gambiae* s.l constituted 66.7% (n = 3675) and 33.3% (n = 1832) were *An. funestus* s.l. *An. arabiensis*, *An.* quadriannulatus and *An. funestus* s.s. were identified. *An. arabiensis* was more frequently observed biting humans outdoors (0.567) than indoors (0.443) while *An. funestus* was observed biting indoors (0.532) more than outdoors (0.468). *An. arabiensis* was resistant to deltamethrin with mortality rates of 90-95% while resistance to deltamethrin (14-42%) and bendiocarb (41-56%) was detected in *An. funestus* s.s. Both species of mosquitoes were 100% susceptible to DDT and pirimiphos-methyl. Pre-exposure of *An. arabiensis* and *An. funestus* s.s. to PBO nullified both pyrethroid and carbamate resistance in populations of mosquitoes tested. Outdoor early biting of *An. arabiensis* may hinder malaria elimination efforts by extending residual transmission of malaria, while the detection of pyrethroid and carbamate resistance mediated by oxidases in vector populations might compromise the protective efficacy of ITNs and IRS using these ingredients.

1427

MODELLING POPULATION DYNAMICS OF THE VECTOR *CULEX PIPIENS* IN THE ATLANTA URBAN ENVIRONMENT

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Members of the *Culex pipiens* complex play a critical role in the spillover of urban abroviruses such as West Nile Virus or St. Louis Encephalitis. Mechanistically understanding the drivers of mosquito population

dynamics at larval stages is critical for better informing predictive models and vector management strategies. Despite this recognized need, there is a paucity of empirically fitted mathematical models explaining variable demographic parameters within the vector's main habitat in the USA: urban roadside catch basins. Here, we show results of a series of interlinked experimental and observational studies performed in the city of Atlanta, GA, to quantify the key life history parameters of *Culex pipiens quinquefasciatus* needed to develop a stage-structured population model predicting catch basin productivity. Parameters needed for such a model included survivorship and time to emergence under different nutrient and detritus levels as well as female fecundity on emergence. Larval experiments under controlled temperature conditions showed that in low nutrient environments, survivorship linearly increases with leaf litter presence (Generalized Linear Model, GLM, -4.2765 ± 0.363; $p < 0.001$). As nutrient availability increases, leaf litter has a positive and quadratic effect on survivorship and a negative effect on time to emergence (GLM, 19.3848 ± 0.9385; $p < 0.001$). The non-linear interaction between nutrients and leaf litter was statistically significant and indicative of an additive relationship between both trophic conditions. Ongoing work is incorporating these results into a stage-structured matrix projection model that will be used to predict the population dynamics of *Cx. pipiens quinquefasciatus* in catch basins. Mechanistic characterization of population dynamics of *Cx. pipiens quinquefasciatus* in catch basins can lead to an improved understanding of mosquito productivity in urban areas and the identification of more effective targets for vector management.

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FLIGHT APTITUDE OF FREE-FLYING MOSQUITOES AS A MEASURE OF LONG DISTANCE MIGRATION BEHAVIOR

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Malaria kills over 500,000 people every year in sub-Saharan Africa alone. During the dry season in the Sahel, surface water required for larval sites disappears, halting mosquito reproduction and bringing malaria transmission almost to a standstill. Recent studies have suggested that both *Anopheles gambiae* s.s and *An. arabiensis* (but not *An. coluzzii*) persist in this region by long-distance, wind-assisted migrations, migrating from locations where breeding occurs year round. Direct evidence of long-distance migrating malaria vectors to date is scant. In other insect taxa, in sub-Saharan Africa and elsewhere, windborne long-distance migration occurs seasonally and facilitates exploitation of renewed temporal resources. Our aim here was to measure flight behavior in free-flying, wild mosquitoes, and evaluate if flight propensity exhibits seasonal variation, in accordance with expected movement to- and from the Sahel. We have adapted an assay originally developed for the study of migratory flights in aphids, to measure flight behavior in a group of 100 mosquitoes of predetermined sex, physiology and origin, housed within a 200x30x30 cm vertical flight chamber. The assay involves the measurement of the total displacement of the mosquitoes, over an 18-hours experiment. Mosquito resting positions were captured by a series of digital photographs taken at intervals of 30 minutes, and displacement was calculated by indexing 'arrivals' and 'departures'. Initial laboratory studies revealed a typical circadian rhythm in flight and sugar-feeding patterns. The assay is currently used with field mosquitoes in Mali. Preliminary results suggest that flight propensity was elevated a few weeks after the first rains (June 2015), consistent with our results using a tethered mosquito flight assay. Comprehensive analysis of these experiments will be presented.

1429

SPATIAL DISTRIBUTIONS OF *ANOPHELES* SPECIES IN RELATION TO MALARIA INCIDENCE AT 70 LOCALITIES IN THE HIGHLY ENDEMIC NORTHWEST AND SOUTH PACIFIC COAST REGIONS OF COLOMBIA

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A proper identification of malaria vectors is essential for any attempt to control this disease. Between 40 and 47 *Anopheles* species have been recorded in Colombia, and 8 species complexes have been revealed in the last decade. An update of *Anopheles* species distribution and its relationship with malaria is required, particularly for newly identified members of species complexes. A cross-sectional entomological study was conducted at 70 localities in the highest malaria transmission areas in Colombia. In each locality, immature and adult mosquitoes were collected. All specimens were determined using morphological characters and Cytochrome *c* Oxidase I sequence gene. To detect natural *Plasmodium* infections, enzyme-linked immunosorbent assay and nested PCR analysis were used. *Anopheles* species distribution was spatially associated with malaria prevalence. A total of 1,736 larvae and 12,052 adult mosquitoes were determined. Thirteen *Anopheles* species were identified. COI sequence analysis suggested 4 new lineages for *An. albimanus* (*An. albimanus* B), *An. pseudopunctipennis* s.l., *An. neivai* (*An. neivai nr. neivai* 4), and *An. apicimacula*. Two members of species complexes were identified as *An. nuneztovari* C and *An. albitarsis* I. Another 7 species were confirmed. Four mosquitoes were infected with *Plasmodium* species: *An. albimanus* B (n=1) and *An. nuneztovari* C (n=3). In Northwest of Colombia, *An. nuneztovari* C, *An. albimanus,* and *An. darlingi* were present in the municipalities with API>35 cases/1,000 inhabitants. In the north of South Pacific coast, with a similar API, *An. nuneztovari* C were widely distributed inland, and the main species in coastal regions were *An. albimanus* B and *An. neivai* s.l. In the south of South Pacific coast, 3 *Anopheles* species were found in municipalities with API=15-88 cases/1,000 inhabitants: *An. albimanus* B, *An. calderoni* and *An. neiva* s.l. In conclusion, in the highest malaria areas, 13 *Anopheles* species and 4 new lineages were found. A DNA barcode analysis allowed the taxonomic identification, particularly for species complexes, and to improve the understanding of their relation with malaria transmission.

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STORM DRAINS AS LARVAL DEVELOPMENT AND ADULT RESTING SITES FOR *AEDES AEGYPTI* AND *ALBOPICTUS* IN SALVADOR, BRAZIL

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Brazil reports the larger number of dengue cases in the world (1.6 million in 2015), and simultaneous transmission of dengue, chikungunya and Zika viruses was firstly documented in 2015. Dengue control in Brazil is based on the search for breeding sites in an area, followed by their elimination or treatment. The failure to identify so-called cryptic locations may hinder

the finding of breeding sites, which can be important in diverse areas and circumstances. We studied the importance of storm drains as *Aedes* larval development and adult resting sites in four neighbourhoods, representing different socio economic, infrastructure and topographic conditions, of Salvador, Brazil. A total of 122 storm drains identified in the four study sites were surveyed twice during a 3-month period (total of 241 inspections), and in 49% of the inspections we observed the presence of accumulated water. Adults and larvae of *Ae. aegypti* were captured in two of the four sites, and adults and larvae of *Ae. albopictus* were captured in one of these two. A total of 468 specimens were collected, 148 *Ae. aegypti* (38 adults, 110 larvae), 79 *Ae. albopictus* (48 adults, 31 larvae), and 241 non-*Aedes* (mainly *Culex spp.)* mosquitoes (42 adults, 199 larvae). The presence of *Aedes* mosquitoes was independently associated with the presence of non-*Aedes* mosquitos and with lower accumulated rainfall during the preceding week. We demonstrated that in Salvador, an epicentre of the recent Zika virus outbreak, storm drains often accumulate water and serve both as larval development sites and as adult resting areas for *Ae. aegypti* and *Ae. albopictus*. These potential key environments for *Aedes* reproduction are located in public areas and are often overlooked by vector control campaigns. Targeting alternative breeding sites for surveillance and control needs to be incorporated into vector control programs in Salvador and other urban areas. Targeted efforts to control *Aedes* mosquitoes in these sites need to be developed and applied. In the long term, we advocate for a better design of storm drains that restrict the accumulation of water – though they still may serve as important adult resting sites.

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COMPLEX INTERACTIONS BETWEEN TEMPERATURE AND DIET IN MOSQUITOES REVEAL NEW INSIGHTS INTO MALARIA TRANSMISSION UNDER PROJECTED CLIMATE CHANGE SCENARIOS

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There is great concern as to how global climate change may affect vector-borne diseases. Numerous studies demonstrate a link between mean temperature and mosquito survival; however, in the field, temperature fluctuates dynamically throughout the day. Furthermore, temperature has been shown to significantly affect mosquito blood feeding behavior. Here, we observed the effect of several temperature regimes on daily mosquito survival. Mosquitoes were divided into six temperature groups: 27°C, 30°C, 34°C, as well as three treatments of the same mean, but allowed to fluctuate a total of 10°C over the course of the day. Females were then further allocated to one of four dietary regimes: 1) water feeding only, 2) sugar feeding only, 3) water feeding with the provision of a human blood meal every three days, or 4) sugar feeding with a blood meal every three days. Across all temperature treatments, blood feeding significantly improved survivorship compared to those maintained exclusively on sugar or water. Females imbibing both blood and sugar experienced the greatest increased survivorship; when compared to those feeding on blood and water, it is clear that sugar consumption provided an additional source of energy. As temperatures increase, the time to complete development of the malaria parasite within the mosquito decreases; these data show that in hotter temperatures, the daily survival rate of *Anopheles stephensi* females would be sufficient to effectively transmit malaria parasites. These results suggest a complex relationship between diet and temperature; not only are blood meals the source of disease transmission, but they also increase vector survival. It is crucial that these intricacies be taken into account when determining the best control programs for individual countries in the face of global climate change.

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INTERROGATING THE ROLE OF STEROID HORMONES IN THE REPRODUCTIVE ECOLOGY OF *ANOPHELES GAMBIAE* MOSQUITOES

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Human malaria is a major public health burden in tropical and subtropical countries and is transmitted exclusively by female *Anopheles* mosquitoes. Malaria control strategies aimed at inducing sexual sterility in natural vector populations are an attractive alternative due to increasing levels of insecticide resistance within medically important anophelines. However, the development of these strategies is hampered by a profound lack of knowledge regarding the most basic elements of *Anopheles* mating ecology. Recently, our group has demonstrated that the suite of mating induced changes in *An. gambiae* females is largely mediated by male transference of the steroid hormone 20-hydroxyecdysone (20E) during copulation. We have also demonstrated that precopulatory female choice is likely predicated upon the differential ability of males to synthesize 20E in their male accessory glands (MAGs). Females accept matings from males that not only have double the titers of 20E relative to their unsuccessful rivals, but also have distinctly different chemical contact cue profiles. In order to investigate this relationship further, we created a line of transgenic *An. gambiae* males that expresses a MAG specific 20E-targeting oxidase that inactivates the hormone. Here we show that this transgenic line of males is deficient in 20E synthesis, as males transfered ~ 70% less 20E to females than control males. Moreover, mating assays revealed that these transgenic males exhibit reduced mating success, with females refusing ejaculate transfer from these males even after forming a mating pair. Through GC/MS analysis of transgenic males, we have also tested the relationship between male 20E titers and chemical contact cue profiles. Taken together, these results provide compelling evidence for the first time that *An. gambiae* females exhibit both pre- and pericopulatory mechanisms of choice, with male 20E being a key factor, a critical insight into the mating ecology of a major disease vector.

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APPLICATION OF BAYESIAN MAXIMUM ENTROPY TO ESTIMATE THE ASSOCIATION BETWEEN ADULT *AEDES AEGYPTI* DENSITY AND SIX-MONTH RISK OF DENGUE VIRUS SEROCONVERSION

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Efforts to characterize *Aedes aegypti* indicators of abundance have not consistently identified associations with increased risk of dengue virus (DENV) seroconversion. This is partly the result of cross-sectional measures of mosquito density in which no mosquitoes are observed when low levels of infestation may truly be present. We created cross-sectional entomological monitoring indicators by incorporating the spatial distribution of adult mosquitoes to estimate the association between mosquito density and DENV seroconversion. Categorical density variables were generated based on the presence of any adult *Ae. aegypti* in an adjacent household as well as households within 30, 50 and 100 meters, respectively. Continuous density was calculated as adult mosquitoes per square meter, per resident reported and per household room surveyed; these measures were also analyzed as categorical variables (comparing ≥0.01 to <0.01). The spatial and temporal covariance of these continuous

measures were then modeled and mosquito density was estimated using the Bayesian Maximum Entropy (BME) geostatistical framework. Spatially-modified densities were used to estimate the six-month risk of DENV seroconversion using log binomial models. Construction of density variables by inclusion of adjacent households resulted in a weak association (risk ratio: 1.10 (95% CI: 0.99, 1.23), but measures within 30, 50 and 100 meters did not demonstrate an increased seroconversion risk. Adult *Ae. aegypti* per household area (RR: 1.01; 95% CI: 0.86, 1.18) and density predictions generated using BME (RR: 1.03; 95% CI: 0.91, 1.16) were not associated with risk. The spatial covariance model suggests that spatial correlation among entomological data occurs at a very fine scale, within approximately 25 meters. The lack of association with DENV seroconversion suggests that the inability of cross-sectional entomological measures of mosquito density to identify individuals at an elevated risk of DENV is not improved by incorporating measurements at various spatial scales.

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QUANTUM DOT LABELLING OF *AEDES ALBOPICTUS* BACTERIA: A NEW METHOD TO STUDY MOSQUITO DISPERSAL BEHAVIORS

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Aedes mosquito transmits important human diseases such as dengue and Zika. Bacterial microbiota-mosquito holobiont plays an important role in various aspects of mosquito life history and vector competence. The life table study using water from natural habitats but depleted with bacteria by filtering or antibiotics showed very high larval mortality in *Aedes albopictus,* demonstrating important role of bacteria in *Ae. albopictus* larval development in nature. Using Miseq pyrosequencing of the 16S rRNA gene V4 hyper-variable region, mosquito bacterial microbiota was examined at different developmental stages from major types of larval habitats. Despite of highly diverse bacterial microbiomes in aquatic habitats and mosquito larvae, *Wolbachia, Aromonas, Novisprillum* and several other genera were the dominant bacteria in adult mosquitoes. We applied the knowledge on the dominant and cultured bacteria in adult mosquitoes to determine whether bacteria can be used as a new labeling method to study mosquito dispersal behaviors. To prove the principle of this method, mannose-modified fluorescent carbon quantum dots (Man-CQDs) were synthesized and used to label *Escherichia coli*. *Aedes albopictus* fed with Man-CQDs labeled *E. coli* showed a constant fluorescence. Larval life table study found that Man-CQDs had low or no toxicity to the mosquitoes. We are currently testing this labeling method with the dominant and cultured bacteria found in natural adult mosquitoes, and conducting field experiments to determine mosquito dispersal behaviors. Mosquito fluorescence labeling through Man-CQDs labeled bacteria may provide a potential new method to explore the function of bacteria in mosquitoes and to study mosquito behaviors.

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MODELING THE IMPACTS OF CO-CIRCULATING HEMOPARASITES IN MOSQUITOES ON WEST NILE VIRUS TRANSMISSION

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Polyparasitism, or the simultaneous infection with two or more parasites in a single host, is a widespread phenomenon that may influence infectious disease dynamics. Because of the abundance of parasitic coinfections in nature, blood-feeding arthropods are often exposed to multiple parasites during a single blood meal. *Culex* mosquitoes, the main vectors of West Nile virus (WNV), may ingest a variety of viral, protozoan, and macro-parasitic hosts found among avian and mammalian bloodmeal hosts. However, the downstream transmission consequences of mosquitoes ingesting these organisms are unknown. SIR-based mathematical models were used to explore outcomes where WNV-hemoparasite co-ingestion altered vector survival, vector competence, the extrinsic incubation period, or vector feeding ecology. We conducted a sensitivity analysis to highlight the parameters with the greatest effect on the reproductive number of WNV. Results highlight the potential for population-level impacts of within-host interactions and identify mechanisms capable of driving fine-scale transmission of WNV.

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MIDGUT COMMENSALS REGULATE INFECTION BY ZIKA AND SINDBIS VIRUSES IN *AEDES AEGYPTI*

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In recent years, mosquito-borne viruses such as Dengue (DENV), Chikungunya (CHIKV), and Zika (ZIKV) have become globally disseminated and are a significant cause of human morbidity and mortality. In order for transmission to occur, viruses must first infect the mosquito midgut and subsequently pass into the hemolymph where they can circulate to infect other tissues. Numerous studies have demonstrated that the mosquito midgut is an important barrier that bloodmeal-acquired viruses must overcome. Therefore, understanding the mechanisms underlying successful infection of the midgut is crucial. The mosquito microbiome plays an important role in shaping the midgut environment and has been previously shown to regulate susceptibility to infection by different arboviruses. To contribute to this body of knowledge, we have tested whether reducing midgut commensal bacteria affects *Aedes aegypti* infection by both Zika virus (ZIKV) and Sindbis virus (SINV). Our preliminary data suggests that depletion of commensals by antibiotics during both of these viral infections results in a decrease in viral transcript levels. We are validating these results by assaying virus at the protein level by western blot and determining localization by confocal microscopy. Specifically, we are interested in exploring the mechanism behind this phenotype. 16S sequencing and CFU plating will be used to define the bacterial signals that elicit infection-permissive responses from the intestinal epithelium. Toll and Imd NF-κB signaling pathways are important for regulation of the gut microbiome. Bacterial products bind to cell surface receptors and activate signaling in the intestinal epithelium. Therefore, we are using RNA-seq to characterize the differences between antibiotic and sugarfed *Ae. aegypti* in activation of these pathways during viral infection. Results from these studies will lead to mechanistic insights that may yield novel strategies to block disease transmission.

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VECTORBASE: THE USE OF THIS DATABASE FOR NEW ANALYSES, DESCRIPTIONS AND HYPOTHESIS TESTING

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VectorBase is a NIAID/NIH-funded bioinformatics resource center for invertebrate vectors of human pathogens. This database has the genomes of vectors of medical importance such as *Anopheles gambiae*, *Aedes aegypti*, *Culex quinquefasciatus*, others in the groups Diptera, Hemiptera, Phthiraptera, Acari and the snail *Biomphalaria glabrata*, the intermediate host of *Schistosoma mansoni*. In the last year the number of genomes hosted has increased to 40, with new additions including *Aedes albopictus* and non-vector but useful key species for comparative genomics of traits of interest such as *Sarcoptes scabiei var. canis*, *Stomoxys calcitrans* and *Cimex lectularius*. In addition to genomes, transcriptomes and proteomes, VectorBase also hosts lab and field collected metadata, genetic variation (*e.g.,* SNPs), expression (microarrays and RNAseq) and insecticide-resistance phenotypes. We will demo how the freely available VectorBase

data can be visualized, browsed and queried, creating the possibility of new analyses, descriptions and hypotheses testing. Thesis or publications using this resource, are kindly ask to reference the paper or papers were the data was originally published and VectorBase most recent paper, as explained in the website under the "Help" navigation tab. We will also demo how to export or download big or small data sets, both for simple and complex queries. Analyses of these data can be performed with the site tools, which include Galaxy, a web-based platform for data intensive biomedical research, or any other external tool. Follow this link for the latest data, tool and resources updates called releases, www.vectorbase.org/releases, send questions or comments to info@vectorbase.org, and visit the site YouTube channel for video tutorials in this link, https://goo.gl/mChdGh.

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ECOLOGY OF LA CROSSE VIRUS (LACV) VECTORS ALONG FOREST-TO-FIELD ECOTONES IN WESTERN NORTH CAROLINA

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La Crosse Encephalitis (LACE) is a pediatric disease with a recent emergence in the number of reported cases in Appalachia. This increase may be due to improved reporting and/or a greater exposure to La Crosse virus (LACv) vectors. The LACv is historically transmitted by the sylvan eastern tree-hole mosquito (*Aedes* triseriatus). However, recently invasive species (*Ae. albopictus* and Ae. japonicus), likely secondary peri-domestic vectors known to co-occur with Ae. triseriatus, complicate the understanding of LACv ecology. The goal of this study was to determine the effect of landscape structure (i.e., forest-to-field ecotones) and artificial container introduction (i.e., tires) on the distribution and abundance of the LACv in western NC. We hypothesized that 1) Canopy-associated environmental variables determine LACv vectors' distribution and clustering along these ecotones; and that 2) Tire introduction increases local (habitat-specific) and overall (across ecotone) abundance of LACv vectors. We ran 2 parallel transects per site (6 sites total), each 200-meters in length, 15 ovitraps per transect; we also deployed traps for gravid (BG Sentinels and Landing-Biting) and resting (Nasci aspirator) mosquitoes. We incorporated 9 tires in each experimental plot: 2 sites received treatment in the field, 2 sites in the forest, and 2 sites served as control. Preliminary results suggest habitat preferences with *Ae. albopictus* more abundant in the field habitats, and Ae. japonicus as well as Ae. triseriatus more common in the forest and edge habitats. The artificial container introduction appeared to increase the abundance of all species, particularly in their "preferred" habitats; however, it did not result in altered oviposition patterns along the ecotone.

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EVALUATION OF KNOWLEDGE AND PRACTICES OF RESIDENTS FOR THE PREVENTION OF MOSQUITO-BORNE VIRUSES IN NEW ORLEANS, LOUISIANA

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To understand local transmission potential for the emergence of arboviral epidemics including Zika, dengue and chikungunya, the abundance of competent vector populations and residential sources should be evaluated. This study assessed the knowledge, attitudes and practices of New Orleans residents regarding mosquitoes and arboviruses and identified frequent breeding habitats on residential properties. Residents indicated frequent mosquito exposure including mosquitoes being a problem in the yard (63.2%), being bitten by mosquitoes frequently (45.9%) and spending time outside in evening daily (37.8%). Central air conditioning was common (70.5%) however, 30.0% reported opening windows frequently and sometimes finding mosquitoes inside the house (54.5%). Property inspections in November and December 2015 yielded an average of 1.4 water-holding containers per residence and a House Index of 31.8. Of the 115 containers surveyed 36.5% were positive for mosquito larvae and 13.9% for pupae. The most common mosquito species was *Aedes aegypti* (85.9%); far less common were Culex quinquefasciatus (11.3%) and *Ae. albopictus* (3.3%). Additional container surveys and questionnaires are planned for May-July 2016, and adult mosquito surveillance will be conducted using BG Sentinel traps. Large urban populations of *Aedes aegypti* and *Ae. albopictus* are present in New Orleans, Louisiana and the potential of introduction of Zika virus by a viremic individual is of great concern. It is essential to identify, educate, and eliminate residential mosquito breeding locations for *Ae. aegypti* on a community-wide level, and the results from these surveys will be used to produce tailored educational outreach materials. The long-term control of arboviral diseases is only possible through an integrated public health approach, rapid case identification, and sustainable vector control strategies.

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VECTORIAL CAPACITY OF *AEDES ALBOPICTUS* ACROSS THE UNITED STATES

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There is accumulating evidence that predicting vector-borne disease transmission is fraught with complexity. Recent work in a variety of transmission systems suggest that climate patterns have important direct impacts on vector-borne disease and define the potential environmental ranges of vector-borne pathogens. However, the realized distribution and extent of vector-borne disease transmission will also depend upon a variety of non-climatic factors, such as genotypic differences in local vector and pathogen populations, as well as socioeconomic and demographic factors that define variation in human exposure. The proposed study will investigate the invasive *Aedes albopictus* (Asian tiger mosquito) - arbovirus transmission system. *Ae. albopictus* is highly abundant, has a large and expanding distribution within the U.S., is a highly competent vector for dengue, chikungunya, and potentially Zika viruses, and has been linked to explosive arbovirus outbreaks in temperate zones.The goal of this project is to identify which environmental and genetic factors contribute to variation in vectorial capacity. To do this we first empirically quantified how fitness and transmission potential vary across the U.S. distribution of *Ae. albopictus* by running large-scale, common garden transplant experiments under semi-field conditions across a latitudinal cline. Using life table analysis, we compared the fitness differences in sympatric vs. allopatric populations to determine if populations are adapted to local environmental conditions and to generate age-specific estimates of key mosquito life history traits that drive transmission (e.g. larval development rates, longevity, fecundity, and biting rates). From this study, we generate age-dependent models of vectorial capacity to predict how lifetime transmission potential varies across latitude and populations of *Ae. albopictus* in the United States.

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DETECTION OF MAMMALIAN ANTIBODIES AGAINST ROSS RIVER VIRUS IN MOSQUITO BLOOD MEALS AND POTENTIAL FOR ARBOVIRUS SURVEILLANCE

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Mosquito-borne alphaviruses are the causative agents of several debilitating diseases that have been associated with large, cross continental outbreaks, as demonstrated recently by chikungunya virus. Ross River virus (RRV) is an alphavirus endemic to Australia and the Pacific which is the agent of a debilitating disease with symptoms including fever,

arthritic joint pain and rash. RRV is characterized by a broad association with a variety of mosquito vectors and vertebrate hosts. A number of these hosts are native Australian marsupials, including kangaroos, wallabies and koalas. The complex ecology of the virus present large challenges for disease surveillance, epidemiology and control. We are developing a novel xenodiagnostic assay strategy to determine the seroprevalence of RRV antibodies among vertebrate host populations. The strategy avoids animal ethics dilemmas by harnessing the natural behavior of resident mosquito populations to sample blood from a wide variety of vertebrate hosts. We demonstrate the ability to detect RRV IgG from within mosquitoes that originate from any vertebrate host species. We are utilizing a population of koalas with a seroprevalence for RRV IgG of 75% and a colony of flying foxes (Pteropus spp.) in a suburb of Brisbane. This work will provide insights and strategies for improved epidemiology of RRV and potentially other mosquito-borne diseases.

1442

TARGETED XENOMONITORING FOR LYMPHATIC FILARIASIS IN HIGH RISK COMMUNITIES AS PART OF POST-MASS DRUG ADMINISTRATION AND ENDGAME SURVEILLANCE IN MALAWI

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Malawi is on track for the elimination of lymphatic filariasis (LF), with more than five rounds of annual mass drug administration (MDA) and successful transmission assessment surveys (TAS) to indicate transmission has been interrupted. Now there is a need to implement post-MDA surveillance strategies, including xenomonitoring to provide further evidence of elimination, especially in high risk communities identified in baseline prevalence surveys. The Neglected Tropical Disease (NTD) laboratory in Blantyre provides essential support to the National LF Elimination Programme, and in 2010-2011 collected and analysed >12,000 mosquitoes from across the country during the initial stages of the MDA programme. Relatively high rates of LF infections by RT-PCR (8%) were found, and the majority of infections were in *Anopheles funestus* from Chikwawa District in the high risk Southern Region of the country. Co-incidentally high levels of pyrethroid insecticide resistance were also reported in this species, which has implication for the effectiveness of additional vector control interventions, especially long-lasting insecticidal nets (LLINs). The aim of this study therefore was to conduct a follow-up post-MDA assessment in the three high risk villages where high mosquito infection rates were found in the initial MDA stages. In addition, an assessment of five highly endemic villages where >10 morbidity cases have been reported during recent mapping activities in Chikwawa and Nsanje will be conducted. The work will specifically focus on collecting mosquitoes using pyrethrum spray catches (PSCs) and window traps over a three month period across 8 high risk villages from >20 individual trapping sites. More than 7000 mosquitoes are expected to be processed, including species identification, examination for *Wuchereria bancrofti* microfilaria infections using RT-PCR and insecticide resistance standard laboratory protocols. This study will help to establish a targeted xenomonitoring protocol in high risk areas and provide information to the LF programme as part of its post-MDA and endgame surveillance strategy.

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TROPICAL DISEASE IN LATE 18TH SURINAM: THE CASE OF CAPT. JOHN STEDMAN, 1772-1777

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Historians of tropical medicine have largely ignored John Gabriel Stedman's The Narrative of a Five Years Expedition against the Revolted Negroes of Surinam (1796). Contemporaries commandeered his writings as a cruelly vivid indictment of slavery in Surinam. Although a mercenary on the payroll of Dutch planters, Stedman himself condemned the brutally that he witnessed daily—observations often redacted from early editions. Stedman's five-year tale of life in Surinam represents far more than a graphic indictment of slavery. Rather, it provides a vivid window into late 18th century tropical medicine in South America. Drawing on Stedman's own first-hand observations, this project will examine his daily encounters with tropical disease, insect vectors, and medical therapy within the context of late 18th century colonial medicine in the Americas.

1444

DIFFERENCES IN PRELACTEAL FEEDING ON THE ISLAND OF HISPANIOLA: THE DOMINICAN REPUBLIC AND HAITI

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Despite sharing the island of Hispaniola, the Dominican Republic (DR) has significantly lower rates of exclusive breastfeeding (EBF) than Haiti in the first six-months of life. Prelacteal feeding (PLF), the use of non-breastmilk feeds in the first three days of life and/or before breastmilk comes in, is common in the DR and is known to undermine EBF. Whether PLF is more common in the DR than Haiti has not been examined in recent data, nor while controlling for other factors that influence PLF (e.g., caesarean sections [C-section]). This study aimed to determine (i) whether PLF differs between the DR and Haiti, and (ii) whether such differences persist after controlling for potential confounding variables. This study used data from the most recent Demographic and Health Surveys from the DR (2013) and Haiti (2012). PLF was found to be much higher in the DR (62.5%) than Haiti (20.3%). Infant formula and other non-breast milks were the most common PLF choices in the DR, but were rarely used in Haiti. In contrast, Haitians more often administered sugar water to newborns than Dominicans. In bivariate analysis, the prevalence of PLF increased as a function of increasing household wealth in the DR, but was unrelated to wealth quintiles in Haiti. In a final multivariate model, being Dominican substantially increased the odds of PLF despite controlling for multiple other variables. Having had a C-section and not having put the child to the breast within one hour after delivery also significantly and independently increased the odds of PLF. Further investigation is warranted to identify what other factors contribute to the significantly higher PLF rates amongst Dominicans versus Haitians. In addition, intervention studies are required to determine approaches to reduce PLF, which may increase EBF rates in the DR and elsewhere.

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POOLING KNOWLEDGE AND EXPERIENCE TO IMPROVE CLINICAL RESEARCH STANDARDS IN LOW- AND MIDDLE-INCOME COUNTRIES: THE EXPERIENCE OF THE SWITCHING THE POLES NETWORK (2008-2016)

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The Switching The Poles Clinical Research Network, started in 2008, brings together 13 research institutions from Belgium, Benin, Burkina Faso, Cambodia, Cuba, the Democratic Republic of Congo, Ethiopia, India, Indonesia, Nepal, Peru, The Gambia and Vietnam. It aims at strengthening partners' capacity to set-up, conduct and lead non-commercial clinical research programs that address the priority health needs of these regions and comply with appropriate ethical and Good Clinical (Laboratory) Practice (GC(L)P) standards. The Network adopted an approach based on practical thematic working groups, that favour the involvement of young researchers and traditionally 'neglected profiles' (*e.g.* data managers, laboratory staff), with the potential of bringing a direct benefit to research projects. The main groups include GCP, GCLP, clinical data management (DM) and clinical monitoring in resource-constrained settings, and informed consent in vulnerable communities. We developed a theoretical and practical approach to teaching GCP and GCLP; a set of standardised DM procedures; and an e-platform (admitnetwork.org) for consultation and peer advice among clinical data managers, who are traditionally quite isolated in small non-commercial research groups. We also started the field coaching of clinical monitors, facilitated South-South collaboration in different aspects of clinical research and took public positioning on research ethics issues, *e.g.* the double ethical review in externally-sponsored trials and the approach to informed consent in socially vulnerable populations. The inclusion of partners from three continents, with different linguistic and cultural features, resulted in cross-fertilization and enrichment, while the small size of the network favored interpersonal collaboration, and it could make some achievements sustainable also in absence of prolonged external funding. Our experience shows that small but multi-cultural networks are flexible, can rapidly adapt to address the partners' needs, and provide an excellent platform for supporting young researchers and promoting South-South collaboration.

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TELEPATHOLOGY FOR RAPID TURNAROUND TIME IN MALIGNANCY DIAGNOSIS IN LOW-MIDDLE INCOME SETTINGS

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Delivering cancer care in low-resource settings is dependent on developing efficient and accurate pathology systems. In this study, we analyzed the efficacy of implementing a telepathology system to remotely provide cancer diagnostics to Butaro District Hospital in rural Rwanda. Our system consisted of static images obtained by histotechnologists using a standard protocol, uploaded to ipath-network.com, and reviewed by a team of pathologists with various areas of expertise in common malignancies. Over the 9-month implementation of telepatholgy, we divided the study into three segments—training, technical workflow, and testing segment. In this presentation, we will breakdown the efficacy of the telepathology system in Butaro District Hospital for oncology cases. For the three implementation phases over the 9-month study period, we will present the turn-around time, from procedure date to result, as well as the volume of cases triaged for pathologist review that were unable to be diagnosed through telepathology. Over the three implementation phases, the turn-around time of cases drastically decreased, allowing clinicians to receive results and initiate more accurate and timely treatment for cancer patients. Simultaneously, the percentage of cases triaged for pathologist review that could not be properly diagnosed through telepathology significantly decreased through improvements in technical imaging, communication, and workflow management.

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EVALUATION OF A HANDHELD MOLECULAR ASSAY AS A RAPID CLOUD BASED POINT OF CARE ASSAY FOR THE FIELD DETECTION OF RESPIRATORY VIRUSES IN KENYA

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Influenza and respiratory syncytial viruses (RSV) are important causes of respiratory morbidity and mortality. Polymerase chain reaction (PCR) is the most reliable diagnostic test, but requires advanced laboratory facilities. Point of care (POC) rapid tests for influenza characteristically have low-moderate sensitivity, varying substantially with age and the time of testing related to illness onset. Rapid and accurate detection of respiratory pathogens in less resourced, often rural settings is key for effective public health interventions. The Biomeme real-time PCR mini thermocycler with iPhone offers several advantages to traditional PCR, including temperature-stable reagents, simplified protocols allowing for use at POC with limited laboratory experience, and instant communication of results. We evaluated the performance of this thermocycler against traditional Center for Disease Control and Prevention (CDC) real-time PCR assays. A total of 119 stored nasal and oral pharyngeal samples were identified, and RNA extracted and tested using both assays. CDC assays detected 35 Influenza-A, 11 Influenza-B, 45 RSV and 28 influenza/RSV-negative specimens. For Influenza A, Biomeme detected 30/35 positives compared with CDC assay, for a sensitivity, specificity and agreement of 86 (95% confidence interval (ci) 74,97), 96 (95% ci 90,100) and 81% (Kappa= 81(95% ci 67,95)) respectively. For Influenza B, Biomeme detected 7/11 positives compared with CDC assay, for a sensitivity, specificity and agreement of 64 (95% ci 35,92), 96 (95% ci 90,100) and 66% (Kappa=66 (95% ci 38,93)) respectively. For RSV, Biomeme detected 37/45 positives compared with CDC assay, for a sensitivity, specificity and agreement of 82 (95% ci 71,93), 100 and 78% (kappa=78(95% ci 64,92)) respectively. Generally, there was good test agreement between Biomeme and CDC assays. Biomeme has potential as an accurate alternative POC diagnostics in sub-Saharan Africa, allowing detection of Influenza and other respiratory pathogens in medical facilities with limited laboratory capacity.

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IDENTIFYING AVENUES IN THE MANAGEMENT OF FEBRILE ILLNESS BY EXAMINING COMMUNITY HEALTH SEEKING PATTERNS

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Malaria remains a major public health problem in Kenya. Early appropriate diagnosis and treatment can prevent severe illness. This depends on the recognition of the symptoms and signs and on treatment actions taken. We describe health seeking behavior for febrile illness in 3 rural malaria endemic areas prior to implementation of a large cluster randomized study. A population based household survey was conducted in western Kenya in a random sample of households. 2,065 participants who reported a malaria-like illness in the past four weeks were enrolled. 21% of respondents used drugs that were available at home as a first action, 53.5% visited drug shops and 24.6% went to a health facility. 44% of participants had a malaria test for the illness, and 82.2% of tests were positive by self-report. Adults (OR 1.29 95%CI: 1.08-1.55), those less educated (OR 1.29 95%CI: 1.08-1.55) and those not employed were more likely to go to drug shops as a first action. More educated participants were more likely to be tested (OR 1.42, 95%CI: 1.22-1.65), but less likely to have a positive test. Overall, 76.4% reported taking an Artemisinin Combination Therapy (ACT), including 53.3% of malaria-negative participants, and 68.6% of participants with no test. 60% of those who took an ACT purchased it from a shop. The odds of taking an ACT did not differ based on whether the participant had a malaria test or whether the test was positive or negative, but the odds of taking an ACT was three-times higher if they purchased drugs in a shop (OR 3.2, 95%CI: 2.6-4.0). 42.9% of those who took action for their illness also took a second follow up action, usually visiting a health facility (72%). Those who initially took drugs available at home and from drug shops were more likely to take a second action (34.74%, 56.21% respectively) as compared to those who had initially visited health facilities (8.7%). Most ACTs are obtained from the private retail sector but targeting of ACTs to those with a confirmed malaria infection is inadequate. This is underscored by the large proportion of people who do not recover after seeking treatment in the retail sector and go on to attend a health facility.

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EXPERIENCES AND CHALLENGES OPERATING AN EBOLA VIRUS DISEASE DIAGNOSTIC LABORATORY IN RURAL LIBERIA

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In August 2014, the World Health Organization declared the Ebola Virus Disease (EVD) outbreak in the West African nations of Guinea, Liberia, and Sierra Leone a public health emergency of international concern. As part of the international response to the outbreak in Liberia, laboratories were opened and dedicated to testing specimens for EVD infection across the nation. These laboratories served to provide rapid and accurate information to assist epidemiologists and other public health workers in controlling the spread of the outbreak by testing specimens from both living and deceased patients thought to be infected with the virus. In February 2015, the Academic Consortium Combating Ebola in Liberia (ACCEL), in coordination with the United States Centers for Disease Control and Prevention and the Liberia Ministry of Health and Social Welfare, began performing EVD testing in Tappita, Liberia. Since that time, the laboratory has processed and tested over 9,000 specimens and continues to be one of four enduring EVD laboratories in the nation. In addition to providing rapid molecular diagnostic capability for epidemiological investigations, the Tappita EVD Laboratory also provides rRT-PCR testing for the semen of male EVD survivors in support of Liberia's Men's Health Screening Program. Although testing for highly pathogenic agents presents inherent challenges, operating in rural Liberia offers unique challenges including further issues with logistics management, workforce development, sustainability, infrastructure improvement, and the implementation of new technologies. This requires both a knowledgeable and adaptable organization. Herein, we present our experiences running one of the largest EVD laboratories in Liberia and the challenges encountered.

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THE COSTS AND IMPACT OF COMMUNITY HEALTH SERVICES IN MALAWI AND SIERRA LEONE

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In Malawi and Sierra Leone, community health workers (CHWs) play a critical role in extending access to health services and addressing priority issues ranging from Ebola prevention to malaria case management. Despite evidence on the benefits of community health services (CHS), there is limited information on the required financing for effective, integrated CHW programs, and, consequently, programs frequently lack long-term financing plans and are under-funded. In February and March 2015, MSH, through funding from UNICEF, piloted a methodology and tool to calculate the costs and the estimated return on investment (i.e. lives saved) of national CHS packages in Malawi and Sierra Leone. Using an "ingredients-based" approach, MSH staff collected actual service and financial data through semi-structured questionnaires administered to 48 CHWs and 22 supervisors in four districts and program managers at all levels of the health system. Following data entry into the tool, MSH health economists conducted cost and impact analyses of the current CHS packages and projections of utilization scenarios up to ten years. Based on preliminary study findings in Sierra Leone, the total recurrent cost per CHW averaged $767.25 and the recurrent cost per live saved was $3,176. The main cost-drivers of CHW program were program management followed by CHW supervision and equipment and medicines. A comprehensive understanding of the costs and impact of CHS packages provides evidence for policy makers and planners to advocate for future funding and allocate financial and human resources based on cost and impact projections. However, to be cost-effective and affordable, CHW programs must be well-utilized and key bottlenecks such as stock-outs of medicines, human resource shortages, and inadequate CHW financial incentives must be addressed. This methodology and tool can be adapted for use in other countries for investment case advocacy, service package planning, resource allocation, and cost-effectiveness and financial gap analyses.

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TRENDS IN SOCIOECONOMIC-RELATED HEALTH INEQUALITY IN RURAL WESTERN KENYA: DATA FROM REPEATED HOUSEHOLD MALARIA SURVEYS 2006-2013

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Socio-economic disparity is well recognized as a barrier to achieving health-related international development goals. Socio-economic data are not always analysed or fully utilised for guiding decisions or monitoring the impact of health interventions and programs. In malaria-endemic western Kenya, we examined the trends in malaria parasitemia, malaria medication usage, and care-seeking behaviour at the household level by socio-economic status. We analysed data from annual malaria cross-sectional surveys from 2006 to 2013 based on systematic, cluster, and stratified sampling of 7,253 households in rural western Kenya. Data collected included socioeconomic status (SES), demographics, malaria parasitemia by microscopy, medication usage and care-seeking behaviour. A composite SES score was created from multiple correspondence analyses of household assets, and households were classified as poor (i.e., lowest three quintiles) or less-poor (i.e., highest two quintiles). The gap in the odds of malaria between poor and less-poor (for all ages) was significant in 2007 (OR=1.86, 95% CI: 1.1–3.1, p=0.016) but not in 2013 (OR=1.2, 95% CI: 0.9–1.6, p=0.331). Overall, the declining equity gap in the odds of malaria from 2006–2013 formed a polynomial curve (R^2=0.99; trend p<0.001). Amongst children aged <5 years, the trend in the odds of malaria gap between poor and less-poor was not significant over the study period (p=0.688). The trends in the inequalities in medication usage (p=0.876) and care-seeking behaviour (p=0.181) were not statistically significant over the study period. In western Kenya, substantial inequalities in health indicators, such as malaria parasitemia prevalence, medication usage and care-seeking behaviour, continue to exist. However, the health inequality gap in malaria parasitemia prevalence has decreased over time. These findings provide evidence that targeted malaria prevention and control efforts can help reduce health inequalities among the poorest households in western Kenya.

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ASSESSING URBANIZATION TRENDS FOR PUBLIC HEALTH: MODELLING NIGHTTIME LIGHTS IMAGERY IN AFRICA: 2000 - 2013

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The growth of urban centers and the transition of rural settlements to urban environments has a broad range of public health implications. Sub-Saharan Africa currently has the world's highest urban growth rate of any continent at roughly 4.2% annually and better understanding the spatiotemporal evolution of this process is key to many geospatial disease modelling and burden estimation efforts. Nighttime lights imagery (NTL) captured by National Oceanic and Atmospheric Administration's satellites offer a unique viewpoint for studying urban trends. These data, available as annual composites from 1992-2013 at 1 km resolution, provide a means for spatiotemporal analysis on a global basis. However, inter- and intra-annual differences between satellites make the raw imagery unsuitable for temporal analysis. The objective of this study was to generate a time series of annual inter-calibrated NTL images (2000-2013) to describe patterns of urbanization in Africa and provide input for studies on the relationship between urban land cover and electrification on malaria transmission. Processing included a regression based procedure for inter-calibration of images from different satellite/years. This method used a 1999 image as a reference and values in all other images were adjusted to match its data range. Subsequently, a weighted 5-year moving average was used to reduce annual variability. Low-light thresholding was also used to remove 'overglow', an exaggeration of brightness in urban peripheries. Urban agglomerations were identified using a region grouping function to detect contiguous lighted pixels. Urban cluster sizes were grouped into 6 categories on a log scale (1-100K km^2) and trends in total area assessed temporally. The maximum size and frequency of agglomerations increased in the 10K–100K and 100–1K km^2 categories for densely and sparsely populated countries, respectively. The processed NTL time series will be made openly available to global health researchers as well as the broader scientific community.

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THE ACT CONSORTIUM AND THE GLOBAL HEALTH NETWORK: COLLABORATING TO PROVIDE AN ONLINE, OPEN-ACCESS, COMPREHENSIVE PHARMACOVIGILANCE RESOURCE FOR THOSE WORKING IN TROPICAL INFECTIOUS DISEASES AND GLOBAL HEALTH

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Drug safety signals may remain undetected in resource-poor settings due to a lack of infrastructure for surveillance, under-developed regulatory oversight and poor access to guidance on research methods. Online resources are available but may be unaffordable. Moreover some websites lack maintenance, or provide guidelines and regulations without support for implementation. Few facilitate interaction about the successes and challenges in assessing harm. More tools, discussion and learning opportunities would advance drug safety research in these areas. The ACT Consortium (ACTc) took a comprehensive approach to antimalarial pharmacovigilance (PV); trials on the effects of repeated exposures of artemisinin-based combination therapies (ACTs), and interactions between ACTs and antiretrovirals; the participatory design of adverse event data collection tools; qualitative exploration of influences on participant safety data reports; and a web-based Drug Safety Repository (www.actconsortium.org). Sharing these experiences and resources should benefit others interested in drug safety in tropical infectious diseases and global health, particularly those struggling to access relevant information. As such, ACTc researchers have collaborated with the online open-access science park, The Global Health Network, TGHN (www.tghn.org), to develop a dedicated space for this field. Aside from creating a comprehensive PV resource of relevant information within TGHN, www.globalpharmacovigilance.org brings useful, high quality, up-to-date external resources together in one place. In addition there are original articles on pertinent PV topics, interviews with experts, and fora for discussing drug safety research in the real world. Links are provided to education and training providers, while new free eLearning courses are being developed in-house. The website coordinators make periodic contact with its user group to explore experiences and needs. By engaging

the website community, this contribution to global drug safety will develop iteratively to improve the practice of safety evaluations in resource-poor settings.

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EVALUATION OF CAPACITY BUILDING FOR LEADERSHIP AND GOVERNANCE IN ORDER TO STRENGTHEN HEALTH SYSTEMS IN DEVELOPING COUNTRIES

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In many countries, teaching the topic of leadership is not part of the medical curriculum, however with the recent Ebola outbreak, it clearly showed how a lack of leadership can be a threat to an entire health system. A training course in leadership was taught to 24 medical doctors from eight sub-Saharan countries. The aim of the project was to evaluate the leadership training course and it is application in the context of health systems strengthening in developing countries. The evaluation study was designed using a qualitative methodology whereby individual interviews with all the participants and trainers from the leadership training course were conducted. Focus group discussions were organised in four countries. Questions from the individual interviews were designed following the Kirkpatrick Model for evaluating training programmes, which include, the reaction to the training; learning uptake; behaviour change; and results of the training. The themes generated from the interviews which were highlighted as important to effective leading and governing of health systems in developing countries were, enthusiasm; being a better leader; need for a change; communication; teamwork; personal development; process of implementation of activities; responsibility and governance. During the focus group discussions the themes discussed were non-governmental organizations and representation at the ministry level. The presenter will explore these themes further and demonstrate subsequent changes following the capacity building initiative. The discussion will include positive reaction from all the trained participants, which has enabled them to keep track of their progress through their actions. Training also determined the essential skills required for a leader in healthcare and how participants are able to apply the knowledge gained during the training to their work setting. Additional factors to improve healthcare services by increasing collaboration with Governments and the MOH in order to increase sustainability of healthcare services were addressed.

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HEALTH CARE WORKER MOTIVATION ONE YEAR AFTER THE EBOLA OUTBREAK IN LIBERIA

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Staff motivation is an often overlooked ingredient of quality health services. After a crisis like Ebola, particular attention should be paid to helping health care workers recover. During the Ebola Virus Disease (EVD) outbreak in Liberia, Redemption Hospital, the only free secondary care facility in the city, became the physical and emotional epicentre of the disease. Twenty health care workers became infected and twelve died. The hospital ceased providing most services and was transformed into a holding unit for suspected Ebola cases. In January 2015, Redemption Hospital resumed comprehensive health services with the support of the International Rescue Committee (IRC). Initially it lacked the systems to maintain proper IPC, physical infrastructure for safe waste disposal, personal protective gear, and a sufficient number of skilled staff. Staff morale had been destroyed by the trauma of the outbreak; many did not return and those that did expressed fear and distress. From the outset, the IRC prioritized the mental and physical safety of staff, in addition to focusing on quality services. A year after the resumption of health services, the IRC assessed perceptions of change and factors affecting motivation for staff at Redemption. The qualitative findings revealed that staff expressed an improved sense of safety coming to work, increased confidence in their ability to deliver quality care to patients, and improved sense of pride and value as individuals. A quantitative survey verified these findings: the most important motivating factors reported by staff included commitment to the job (94%), smooth working relationship among staff (86%), and availability of PPE (83%). The least significant factors were monthly salary (27%) and transportation to work (31%). While monetary incentives are important, these findings show that they are not the primary motivator for health care workers at Redemption Hospital, a year after it resumed full services after being closed due to Ebola.

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ESTIMATING THE POTENTIAL DEMAND OF A DENGUE VACCINE TO INFORM VACCINE INTRODUCTION IN THE YUCANTAN PENINSULA: CASE STUDY FOR MEXICO

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Dengue has become a disease of growing importance in Mexico with disease prevalence in endemic areas as high as 80%. In 2015, Sanofi Pasteur's Dengvaxia® became the first dengue vaccine to be licensed for use in Mexico in the 9-45 year olds. As governments at the national and state levels discuss and plan strategies in response to dengue fever and the industry develops and prepares to scale up the production of vaccines, there is a need for estimates of the demand for dengue vaccine and for insights on where the introduction of the vaccine would have strong social and economic impact. In this perspective, strategic demand forecasts can be used to accelerate vaccine access by providing stakeholders with vital information on supply, potential demand, and vaccine costs of vaccine introduction scenarios. Demand estimates will be presented to accelerate vaccine access in the Yucatan peninsula. We will estimate the potential demand, costs, and impact of introducing a dengue vaccine in the Yucatan peninsula based on key stakeholder preferences for 35-year analytic horizon. The Yucatan Peninsula is formed by three states (Yucatan, Campeche and Quintana Roo) in Mexico where dengue fever is on the increase with 2,117 and 2,990 confirmed cases, 8,459 and 31,559 probable cases and 729 and 785 hospitalized cases reported in 2014 and 2015, respectively. We developed an Excel-based model to estimate the potential demand in the Yucatan peninsula from the public and private healthcare perspectives. Introduction scenarios are developed along with model algorithms to model the 35-year analytic horizon based on stakeholder interviews. Model assumptions are derived from government, funders and industry stakeholder interviews and from administrative and surveillance data produced by the Federal Government of Mexico.

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ESTIMATING THE POTENTIAL DEMAND FOR DENGUE VACCINES IN HONDURAS AND PARAGUAY: PRELIMINARY FINDINGS

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Honduras and Paraguay, both Latin American countries with a national GDP per capita below $US31 billion, continue to report growing incidence of and morbidity attributed to dengue fever. The increasing burden of dengue in these countries and the recent approval of the first Dengue vaccine, Dengvaxia®, in neighboring Latin American countries

has highlighted the need for country-specific, equitable dengue vaccine introduction strategies. Currently, there are no studies that quantify the demand and supply of the dengue vaccine to inform the value of vaccine introduction in Honduras and Paraguay. Strategic demand forecasts are a decision-making tool that can evaluate the temporal demand for vaccination, estimate the costs associated with vaccine implementation strategies, and approximate the funding requirements for the vaccine according to different introduction scenarios. This study aims to generate new evidence on the potential demand for two dengue vaccine candidates. We adopt an existing strategic demand forecast model to assess the potential demand for the two vaccine candidates and estimate the implementation costs and health impact of this demand in Honduras and Paraguay. Preliminary findings about the potential demand for dengue vaccines in Honduras and Paraguay will be presented based on extensive stakeholder consultations and a number of vaccine introduction scenarios. Results will rest on country- and state-specific sociodemographic characteristics, dengue epidemiology, price, supply constraints, and timing of licensure. Understanding the potential demand for and associated impact of a dengue vaccine can help develop a viable vaccine introduction strategy, which can significantly accelerate vaccine introduction and decrease the time it takes for countries to begin vaccination following licensure.

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A THRESHOLD ANALYSIS OF THE COST-EFFECTIVENESS OF A DENGUE VACCINE PROGRAM IN YUCATÁN, MEXICO

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The past few months have been pivotal in the effort to prevent and alleviate the burden of dengue fever worldwide. Mexico, Brazil, the Philippines, El Salvador and Paraguay have approved the first dengue vaccine - CYD-TDV, developed by Sanofi Pasteur, paving the way for vaccination campaigns in other endemic countries. The Philippines began its first public, school-based dengue immunization program in selected high-risk areas. Several other dengue vaccine candidates are currently in clinical development. Yucatán is one of Mexico's state that is most challenged with the burden of dengue with an annual incidence rate of 70.89 per 100,000 person-years in 2015, compared to the national average of 22.04 per 100,000 person-years. The cost of introducing the dengue vaccine in this region is likely to be significant. It is important to examine the level of cost per unit of outcome below which this new vaccine might be described as cost-effective to inform vaccine introduction policy. We use a decision tree model, embedded in a strategic demand forecast model, to estimate the incremental cost-effectiveness of using the new dengue vaccine program compared with the status quo, over 5-, 10-, and 15-, 20- and 30-year periods from a government perspective. Standard acceptability curves will be constructed to represent uncertainty around the incremental cost-effectiveness ratio decision rule, and to make cost-effectiveness results comparable to opportunity costs resulting from other health care strategies. Initial results about the cost-effectiveness threshold of a dengue vaccine program in the Yucatán region will be presented and analytic issues will be discussed. The results of the threshold analysis will highlight the importance of threshold values in dengue vaccine introduction decisions.

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TREATMENT AND REFERRAL OF SICK CHILDREN PRESENTING WITH ILLNESSES AT PRIVATE HEALTH CARE FACILITIES IN UGANDA

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Objectives: The main objective of this study was to assess treatment and referral practices for sick children seen at private health care facilities in order to explore ways of improving quality of care in this sector. Methods: A survey was conducted within 57 geographical areas (parishes) from August to October 2014 in Mukono district, central Uganda. Data was collected using a structured questionnaire supplemented by focus group discussions and key informant interviews with community members and private providers. Results: A total of 241 private health facilities were surveyed; 170 (70.5%) were registered drug shops, 59 (24.5%) private clinics and 12 (5.0%) pharmacies. The majority of facilities were selling artemesinin-based-combination therapy, (>96%), Amoxicillin (>90%), Zinc tablets (>77%) and oral rehydration salts, (>76%) all important in treating malaria, pneumonia and diarrhoea among children. Few drug shop (7.1%) and some private clinics (33.9%) had guidelines on integrated management of childhood illnesses. Similarly, only a few drug shop vendors (17.6%) and staff at private clinics (15.3%) knew that amoxicillin was the first-line treatment for pneumonia. Overall, 104/241 (43.2%) of the private health facilities reported that they had referred sick children to higher levels of care in the two weeks prior to the survey. The main constraints to follow referral advice by caretakers were: not appreciating the importance of referral, gender-related decision making and negotiations at household level, poor quality of care at referral facilities, inadequate finances at household level; while the perception that referral leads to loss of prestige and profit was a constraint at facility level, Conclusion: In conclusion, the results show that treatment and referral of sick children at private health facilities faces many challenges. Thus interventions to address constraints to referral of sick children are urgently needed.

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THE CARTER CENTER INTERNATIONAL HEALTH PROGRAM REVIEWS—A UNIQUE MODEL TO ASSESS PROGRAM PROGRESS, CHALLENGES AND IMPROVE PERFORMANCE

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The Carter Center has provided assistance to national programs to interrupt transmission of Guinea worm disease (dracunculiasis) since 1986. The collaborative effort of The Carter Center, ministries of health, WHO, CDC, UNICEF and other partners has reduced cases from an estimated 3.5 million in 1986 to 22 in 2015. The assistance provided by The Carter Center included development of operating procedures and monthly monitoring and reporting of program indicators. These requirements emphasized the need for all components of the programs to be accountable, and programs to be accountable to each other and to partner organizations and donors. In order to keep all staff informed of the status of the national eradication campaign, it became necessary to hold national Guinea Worm Eradication Program (GWEP) reviews, as well as an international review for national programs to report on the status of eradication efforts to partners and donors. This unique forum allows

partners to convene to peer review program progress, discuss challenges and make recommendations focused on improving performance and achieving the goal of eradication. Since its inauguration in Atlanta, GA in 1986, the program review has become the model used to measure program progress and inform programmatic decisions by both The Carter Center and national GWEPs. By 1996 the review had expanded to over 150 participants from 20 countries. In 2006 the review included representation from 21 countries, after the establishment of a separate program in Southern Sudan.

Due to its impact on successful program implementation, the program review model has been adopted by other Carter Center health programs. In 2016, the Center hosted 5 program reviews at its headquarters. While the review has evolved over the past 30 years, it remains an integral part of programming that has informed the Carter Center GWEP as it has helped stop transmission of Guinea worm disease in 17 countries in Africa and Asia. With 4 endemic countries (Chad, Ethiopia, Mali, South Sudan) remaining, The Carter Center will continue to coordinate review meetings at the national and international levels until eradication is achieved.

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LAND COVER MAPPING FOR CONTINENTAL AFRICA

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Land cover type influences transmission of a number of diseases, including vector-borne diseases such as malaria. However, high spatial resolution land cover data through time are lacking for continental Africa, hindering the ability to model and test hypotheses. The objective of this study was to develop a high spatial resolution (30 meter) land cover dataset for continental Africa for the years 2000 and 2015. To generate gold standard model data, high resolution satellite imagery was visually inspected and used to identify (7212 sample points) Landsat pixels that were entirely made up of 1 of 7 classes (water, impervious surface, high biomass, low biomass, rock, sand and bare soil). For model validation purposes, 80% of points from each class were used as training data, with 20% withheld as a validation dataset. Cloud free Landsat 7 and 8 annual composites for 2000 and 2015 were generated. Spectral bands from the Landsat image were then extracted for each of the training and validation points and a random forest model using the full dataset was used to classify the 2000 and 2015 Landsat images into each of the 7 classes. In addition to the Landsat spectral bands, spectral indices such as normalized difference vegetation index (NDVI) and normalized difference water index (NDWI) were used as covariates in the model. Additionally, calibrated night time light imageries from the National Oceanic and Atmospheric Administration (NOAA) were included as a covariate. Using the validation dataset, classification accuracy including omission error and commission error were computed for each land cover class. Model results showed that overall accuracy of classification was over 90 percent. This high resolution land cover product developed for the continental Africa will be available for public use and can potentially enhance the ability to test models and hypotheses.

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USING TREATMENT SEEKING DATA TO DEFINE HEALTH CATCHMENT AREA MODELS: EVIDENCE FROM ZAMBIA

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While routine health facility outpatient data are a vital source for tracking numbers of clinical disease cases in space and time, they are an imperfect measure of disease incidence in communities. Two main difficulties arise when trying to estimate community level incidence from cases reported at the health facility level. First, health records are only representative of those individuals who sought treatment. Thus, the number of cases captured and recorded via health facilities is likely to be an underestimate of the actual number of cases. Second, as information on the location of residence of cases is often lacking, catchment boundaries and populations are often uncertain. A better understanding of the drivers of treatment seeking, as well as the spatial distribution of the patients attending a health facility can help better estimate true incidence rates. Using data from eight rounds of parasite censuses amongst a population of over 300,000 in Southern Province, Zambia, where information on health facility choice and residence location of those seeking treatment for fever was collected, we define a probabilistic model that encodes the decision process of an individual when seeking for treatment and choosing a health facility to attend. Our model factors travel time (based on travel distance and maximum speed allowed by the terrain) as well as the types of health facilities in close proximity (Hospital, Health Center or Health Post). Results demonstrate a negative relationship between travel time to the closest health facility and the decision to seek treatment. Results also demonstrate that individuals are sometimes willing to undergo longer travel times to receive treatment at a specific type of health facility, rather than going to the closest facility available. The model allows for overlapping weighted catchment areas to be defined for each health facility depending on its type, travel time and location of other facilities. This catchment area model will be used in future geospatial modeling work to develop high resolution estimates of the incidence of malaria infection across Zambia.

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A CROSS-SECTIONAL SURVEY OF PERCEPTIONS RELATED TO SYMPTOMS OF MALARIA, CURABLE REPRODUCTIVE TRACT INFECTIONS AND ASSOCIATED TREATMENTS AMONG PREGNANT WOMEN AT HEALTH FACILITIES IN TANZANIA

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Malaria and curable sexually transmitted/reproductive tract infections (STIs/RTIs) in pregnancy are unacceptably high in sub-Saharan Africa due, in part, to poor coverage of antenatal interventions. Investigating the perceptions about these infections and related treatments among pregnant women may help to identify key barriers and facilitators to care-seeking and care provision. A cross-sectional survey of 397 pregnant women was conducted in rural Tanzania to examine perceptions of risk related to symptoms of malaria infection and curable STIs/RTIs, and associated treatments. Overall, 52% of pregnant women reported having a febrile episode in the last four weeks, of whom 79% received

antimalarial treamtent, this despite only 46% of them being diagnosed with malaria. Fever during pregnancy was considered somewhat, very, or extremely harmful by 11%, 25%, and 34% of pregnant women, respectively, whereas 7%, 7%, and 10% believed fever to be not at all harmful, slightly harmful, or somewhat harmful, respectively. Over 20% of participants did not know if malaria treatment was harmful. In the previous four weeks, 53%; 41%; 13% and 9% of pregnant women reported experiencing symptoms of curable STIs/RTIs - lower abdominal pain, genital or vaginal itchiness, vaginal discharge with fishy smell, and genital or vaginal ulcers - respectively. Only 24%, 27%, 33%, and 26% of these women received treatment for their STIs/RTIs symptoms, respectively. The public health implications of these results are evident. Although between 65-70% of pregnant women recognise the potential harm of malaria infection and curable STIs/RTIs in pregnancy, 20-25% of women do not know, or do not believe, that these infections may be harmful to their pregnancies. Malaria treatment is given too commonly without diagnosis, and only one-quarter to one-third of pregnant women with symptoms of curable STIs/RTIs are treated.

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CURABLE REPRODUCTIVE TRACT INFECTIONS: A CROSS-SECTIONAL SURVEY OF PERCEPTIONS RELATED TO SYMPTOMS AND ASSOCIATED TREATMENTS AMONG HEALTH-CARE PROVIDERS AT HEALTH FACILITIES IN TANZANIA

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A cross-sectional survey was conducted among 131 health-care providers in rural Tanzania to examine the knowledge and availability of treatment for syndromes related to curable sexually transmitted and reproductive tract infections (STIs/RTIs) in pregnancy: (1) lower abdominal pain (gonorrhoea and chlamydia), (2) vaginal discharge (bacterial vaginosis, trichomoniasis and yeast infection), (3) genital or vaginal ulcers (syphilis, gonorrhoea, chlamydia, and chancroid), and (4) genital or vaginal itchiness (yeast infection). Perceptions of harm attributable to these syndromes, and to treatment, were recorded. Nine of ten providers believed lower abdominal pain, vaginal discharge, genital or vaginal ulcers were extremely harmful to mothers (89%, 93%, and 95%, respectively); three-quarters (78%) responded similarly about genital or vaginal itchiness. Comparable proportions of providers said syndromes 1-3 were extemely harmful to unborn babies (88%, 94%, and 90%, respectively); three-quarters (76%) considered symdrome 4 to be extremely harmful. Nearly one-third of providers reported that the treatment of lower abdominal pain, vaginal discharge, genital or vaginal ulcers was harmful to mothers (32%, 30%, and 32%, respectively); whereas one in six (16%) providers said treatment of genital or vaginal itchiness was harmful to mothers. Similar proportions of providers reported syndromes 1-3 would be extremely harmful to unborn babies (31%, 35%, and 33%, respectively); one in five (19%) providers believed that treatment of syndrome 4 was harmful to unborn babies. Treatment for these four syndromes was available in 59%, 65%, 46%, and 47% of facilities, respectively, but only one-quarter of 397 pregnant women at the same facilities who reported having an STI/RTI syndrome in the previous four weeks received treatment. These findings suggest that reducing the burden of curable STIs/RTIs during pregnancy will, in part, require investment in retraining to reduce the occasions when some providers may withhold treatment of curable STIs/RTIs out of concern that treatment may be harmful to mothers or unborn babies.

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MAPPING MEASLES IMMUNITY GAPS AT THE SUBPROVINCIAL LEVEL IN THE DEMOCRATIC REPUBLIC OF THE CONGO (DRC) USING 2013-2014 DEMOGRAPHIC AND HEALTH SURVEY (DHS) DATA

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Disease surveillance in the Democratic Republic of Congo (DRC) is logistically challenging due to years of debilitating civil war, insufficient funding and poor infrastructure. Under resource-limited conditions, it is crucial to identify high-priority regions within the country where vaccination efforts should take precedence. We mapped measles immunity gaps throughout the country by geographic survey cluster using data obtained from the 2013-14 DRC Demographics and Health Survey (DHS) with ArcGIS 10.2 software. To estimate measles antibody seroprevalence within areas that were not surveyed during the DHS, we produced a smoothed, interpolated prediction surface to visualize possible differences in measles immunity at the subprovincial level. "Hotspot" spatial clusters of low measles immunity within the country were identified through a Kulldorff spatial scan statistics analysis. Both the interpolated surface map and the spatial scan analysis identified southern Kasai province and western Lualaba province as the most significant hotspots of low measles immunity within the DRC. This study demonstrates that the use of geostatistical mapping methods can be a useful tool for the DRC Expanded Program on Immunization to identify specific areas at the subprovincial level that should be of the highest priority for future measles catch-up campaigns, and offers a novel alternative strategy compared to previous efforts which have treated heterogeneous populations within large administrative areas with the same treatment.

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EBOLA VACCINATION KNOWLEDGE, ATTITUDES, AND BEHAVIOR AMONG HIGH-RISK HEALTH CARE AND FRONT LINE WORKERS, DECEMBER 2014 - JANUARY 2015

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The Centers for Disease Control and Prevention's (CDC) sponsored a phase 2/3 clinical trial of the candidate rVSV-ZEBOV vaccine that was conducted with the College of Medicine and Allied Health Sciences (COMAHS), University of Sierra Leone and the Ministry of Health and Sanitation (MoHS). CDC and MoHS approved the Sierra Leone Trial to Introduce a Vaccine against Ebola (STRIVE) with provisos that 1) communications around the vaccine and trial, including rumor control, were carefully managed and 2) clinical trial activities not impede or conflict with any outbreak response activities. Formative communications research was conducted In December 2014 - January 2015, focusing on health care and frontline workers and the general public to ascertain their understanding and acceptance of an experimental Ebola vaccine. The research was a mixed-methods approach consisting of 1) in-depth interviews with 31 public health official and decision-makers, 2) 35 focus group discussions (FGD) with a total of 316 participants from the target populations,

and 3) a survey of 146 health care and frontline Ebola workers. The findings of the formative research were used to inform a field based communications strategy that focused on three objectives: 1) to build trust in the community by proactively countering potential inaccurate negative perceptions that could impact both the success of the trial and larger response efforts; 2) providing culturally understandable information about the vaccine and STRIVE to potential participants so they could make informed decisions about participation in the trial; 3) to ensure communications supported human subjects protection throughout the trial. We will present the formative research results as well as how that research was applied to the communications strategies and materials for STRIVE. The success of the communication strategy was demonstrated both by the support STRIVE received from the community and by the absence of any sustained negative public concern about the study. This success contributed to the enrollment and vaccination of ~8,000 high-risk health care and frontline Ebola response workers.

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OUTBREAK-RELATED ANXIETY AND PUBLIC POLICY: THE CASE OF EBOLA VIRUS DISEASE AND POLICY PREFERENCES

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Public health officials rely on state-citizen communication and public cooperation to maximize success of emergency preparedness and response endeavors. The role of the public's emotions, particularly fear and anxiety, in behavior and political support during an outbreak is under-investigated yet may yield useful insights for infectious disease policymaking. Data from a nationally representative survey of 1,425 Americans conducted in December 2014 were analyzed using multiple ordered logistic regression models to determine whether fear-based claims about the 2014 West Africa Ebola outbreak, as opposed to claims appealing to morality and human rights, will increase support for the Obama administration's proposed $6.2B appropriations to fund disease control measures. Additional analyses were performed to determine whether fear-based claims have the additional effect of increased support for more exclusionary policies, such as quarantine, isolation, deportation, flight bans, and the destruction of personal property. Respondents' self-rating of both higher anxiety and sadness was positively associated with support for emergency Ebola appropriations. Identification as Republican and conservative was negatively associated with support for Ebola appropriations. For respondents who considered the likelihood of an Ebola outbreak in the next 12 months to be high, there was a positive association with support for appropriations. Respondent belief that Ebola is dangerous was positively associated with support for appropriations. Support for a 21-day quarantine for health workers returning from West Africa was negatively associated with support for appropriations. Support for Obama's executive order suspending deportation orders for undocumented immigrants was positively associated with support for appropriations.

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EBOLA SURVIVOR NETWORKS IN WEST AFRICA: A STRUCTURED APPROACH TO ESTABLISHING EBOLA SURVIVOR SUPPORT NETWORKS AS AN EFFECTIVE COMMUNITY ENGAGEMENT STRATEGY TO HELP FIGHT FUTURE EBOLA EPIDEMICS

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West Africa was disproportionately affected by recent Ebola outbreak. Meaningful engagement of Ebola survivors is increasingly important in the prevention, care and support of Ebola patients in future Ebola epidemics. Engaging with Ebola survivors may speed the translation of discoveries into improved health outcomes. Ebola survivor support networks provide a non-traditional route for authentic engagement of Ebola survivors, patients and communities. As a follow-up to our clinical trial of plasmapheresis of convalescent plasma to treat Ebola victims in West Africa, Clinical Research Management in 2015 began testing new approaches for community engagement which led to the establishment of Ebola Survivor Support Networks in Liberia, Sierra Leone and Guinea. This structured program facilitated project-specific input from governments, communities, academic research institutions and Ebola survivor stakeholders. Peer-to-peer approach was used to recruit, train and organize survivors into networks. The networks were registered as legal entities by respective governments. Basic demographic data of the survivors was systematically collected and stored and a registry of Ebola created for Guinea, Liberia and Sierra Leone. The networks set up offices to support operations. Developers prepared networks to engage with stakeholders and facilitated regular in-person and virtual meetings. A total of six (6) Ebola Survivor Networks were registered. The networks opened national offices in Liberia, Guinea and Sierra Leone. A total of 2800 Ebola survivors were recruited into the Networks and one registry of Ebola survivors created in each country. The Networks engaged 15 research community stakeholders. Participating researchers, reported that partnership with Networks was valuable and that the Networks helped to determine project feasibility and enhanced research implementation. Stakeholders found the Networks to be an acceptable method of engagement. A tool kit was developed to replicate this model and to disseminate this approach.

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THE IMPACT OF CLUSTERING OF UNVACCINATED INDIVIDUALS ON RISK OF OUTBREAKS

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The foundation of efforts to control and eliminate vaccine-preventable diseases like measles rely on estimates of the effective reproduction number (R) and critical vaccination threshold (Vc). Commonly calculated as R = R0*(proportion susceptible), we assume evenly-mixed populations. However, in real populations this assumption fails, and thus true outbreak risk is likely underestimated. We developed a novel methodology to produce an R that captures heterogeneity through scaling the R estimate by the relative probability of susceptible individuals being in contact with one another. We used data available through Demographics and Health Surveys (DHS), which are conducted every 3-5 years in over 90 countries (dhsprogram.com). We developed methods to estimate the clustering of susceptibles that accounts for the clustered sample structure of these data. Applying these methods to Zambia and Tanzania to examine the impact of clustering and its effects on the 2009-10 outbreak in Zambia, we found Tanzania to have a relative level of clustering of 1.37 times that of Zambia (95% CI = 1.04-1.89). We found a ratio 0.96 comparing the estimated R of Tanzania and Zambia. Thus, while Tanzania experienced greater clustering, they counter with higher overall vaccination, resulting in comparable outbreak potential to Zambia. While measles attack rates in Tanzania were relatively constant (mean (SD): 9.3(10.5) per 100,000), in Zambia they were highly variable (mean (SD): 51.2(81.8) per 100,000). This contrast might be partially the consequence of the vaccination and clustering levels, whereby Tanzania experienced more frequent, smaller outbreaks, possibly from higher clustering, while Zambia had a large, population-wide outbreak from lower vaccination coverage and a build-up of susceptibles during non-epidemic years. Our novel approach accurately quantifies the increasing potential for measles outbreaks with increasing clustering of unvaccinated individuals, and our model provides an accessible method to estimate this outbreak potential.

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FAMILY PLANNING IN THE DEMOCRATIC REPUBLIC OF CONGO: UNWANTED PREGNANCY AND ASSOCIATED SOCIODEMOGRAPHIC CHARACTERISTICS

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Unintended or mistimed conceptions, collectively referred to as unwanted pregnancies, are associated with adverse outcomes for both the mother, as they may not be in optimal health for childbearing, and infant, as prenatal care may be delayed. As factors such as educational attainment and socioeconomic status may impact knowledge and attitudes regarding family planning practices, a clear understanding of both the determinants and effects of unmet contraceptive needs is indispensable in low resource settings such as in the Democratic Republic of Congo (DRC). Using 2013 Demographics and Health Survey (DHS) cross-sectional data, we assessed associations between sociodemographic characteristics and select health outcomes with family planning history and attitudes for 18,716 female respondents 15-49 years of age. Of 1164 women reporting an unwanted pregnancy, 75% were not using contraception of any kind, citing breastfeeding (25%), postpartum amenhorrhea (20%), and unknown source for contraceptive attainment (17%) as the most common reasons for nonuse. Unwanted pregnancy was positively associated with maternal age, birth order, and number of children under 5 years of age, while inversely associated with maternal education. Interestingly, head of household sex was also associated with desire for pregnancy, and 706 respondents reported a female head of household. In these preliminary analyses of DRC-DHS nationally representative data, we identified associations between sociodemographic variables and pregnancy intention. Shedding light on the family planning landscape can help to inform public health policy, and programming and, if assessed over time, may indicate changing societal attitudes regarding planning practices. Additionally, unintended and unwanted childbearing can have negative health, social and psychological consequences in children; therefore it is of great importance to assess relationships between pregnancy intention and child health indicators. Such investigations may identify vulnerable subgroups of the population to whom family planning and other public health services may be targeted.

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THE GLOBAL HEALTH EXCHANGE FELLOWSHIP: A PILOT PROGRAM

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The Global Health Exchange Fellowship was a six month pilot project aimed at making global health real through experiential learning for UK and Kenyan trainees in General Practice/Family Medicine and Public Health. The multi-professional and multi-cultural team had two consecutive placements in areas of deprivation, in a low income and high income country. The first was within a rural Maasai community in Kenya, and next was an inner city area in the UK. Using Qualitative research methods, a health needs analysis was carried out in each community. Challenges to health, including socio-economic determinants, were identified and organised into themes by the fellows. These themes were prioritised by the communities using an innovative voting methodology developed by the fellows. Findings were presented to the local health authorities with the aim of informing resource allocation to improve health and reduce inequalities. and fed back to the communities. The Capability Approach was incorporated to encourage community ownership of solutions. Access to healthcare was voted as the number one priority in the rural Maasai community while Education was the top priority in inner city UK. Surprisingly there were a number of similarities in the results from both communities. For instance, Gender Inequality and Culture gave us significant concern as healthcare professionals, however these themes received the fewest votes in the "Very Important" category in Kenya (a low income country) and in the UK (a high income country). The Fellowship was a true exchange between a diverse team of healthcare professionals in terms of knowledge, location, culture and experience. Through their participation, the fellows experienced remarkable personal and professional development. We learned that the challenges to health facing deprived communities globally are complex but similar, and require context specific solutions which take into account social determinants like culture and poverty. This calls for improved interdisciplinary collaboration to improve health and reduce inequalities.

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FACILITATING TRAVEL READINESS IN A GLOBAL ORGANIZATION: SUCCESS OF A PILOT

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Our developmental organization has approximately 18000 employees in 180 different countries globally and is known for a high number of staff members traveling away from their duty-stations, known as mission travel. Staff based in Washington DC have access to a full service travel clinic, used by all our staff, however staff based out of other locations have less access, although are covered under our insurance. In 2015, a travel pilot was launched based out of Johannesburg and Pretoria, South Africa to assess the impact of having similar access to travel medicine preventive care. South Africa was chosen as an appropriate location for the pilot because of the size of the offices as well as the fact that most of the travel taking place is to Sub-Saharan Africa where there are a number of tropical risks not present in South Africa. Data was analyzed from the health risk survey performed in 2014 to assess both knowledge as well as staff habits with respect to pre-trip travel vaccination as well as utilization of malaria prophylaxis. An active intervention was staged over 4 months, with an onsite doctor, education campaigns and travel kits being provided. At the end of 4 months, clinical data was analyzed and staff were surveyed for change in behavior. Significant change was seen in malaria prophylaxis usage as well as increased vaccination. Staff satisfaction was increased greatly as well. This pilot served to demonstrate the value of an active intervention campaign in a high-risk traveling population. Although the staff had coverage for care under their insurance, ease of access helped to facilitate greater coverage and change in behavior. With one on one consultation providing significant value in persuading staff as to the usage of malaria prophylaxis.

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REDUCING HEALTH DISPARITIES IN REFUGEES FROM SUB-SAHARAN AFRICA ENTERING THE UNITED STATES FOR RESETTLEMENT: RETROSPECTIVE REVIEW

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During 2014 there were an estimated 60,000,000 refugees globally. 18,000,000 or 30% of this population was in Sub-Sahara Africa (SSA).

Approximately 75,000 or 0.42% from Sub-Sahara Africa were resettled in the United States. Peer reviewed medical literature and evidence based research for the past two decades described the exposure of refugees to the detrimental effects of pathogenic parasites from countries of origin or while in refugee camps. The aim of the Study is to contribute to a reduction of health disparities that may challenge the effective implementation of interventions and strategies to reduce or eliminate asymptomatic and reactivated diseases. Misdiagnosis of endemic diseases in Sub-Saharan refugees arriving to settle in the United States was also included. We examined peer reviewed medical literature and evidence based research from 2002-2015 using: National Vital Statistics System (NVSS), MEDLINE, PubMed, Cochrane Library, and Science Direct. Data on prevalence of asymptomatic or reactivated pathogens as well as clinical misdiagnosis amongst refugees entering the United States to settle was included in the search. Five medical conditions were selected: Latent TB, Malaria, Schistosomiasis, Strongyloidiasis and Oral health. The criteria applied were: Eosinophil count, regional diagnosis disease, geographical analysis, mapping, screening and surveillance. Although the population of Sub-Saharan refugees entering the United States is small (0.42% or approximately 75,000) findings of the Study indicate that hospital departments are concerned with the economic burden of treating refugees and immigrants. Hospitals may lack health care professionals with sufficient training and skills to treat refugees with tropical or endemic disease. Medical schools must include differential diagnosis in their syllabi that train students to: diagnose, treat and manage the health of the growing foreign born population in the United States that will become patients before or after resettlement. This requires skills and knowledge of diseases endemic to country of origin and risk to the foreign born resettling in the United States. Public Health policy and surveillance does not always include preventative health initiatives or programs that consider the health disparities of refugees born in Sub-Sahara Africa.

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RELAPSES VERSUS REINFECTIONS: ASSESSING THE PARASITOLOGICAL AND CLINICAL IMPLICATIONS USING *PLASMODIUM CYNOMOLGI* AS A MODEL FOR *P. VIVAX*

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Plasmodium vivax causes significant morbidity and mortality worldwide and remains a major obstacle to global malaria eradication. One of the obstacles this parasite presents is its liver-stage reservoir comprised of hypnozoites that are capable of reactivating and causing relapses. Relapses are thought to contribute significantly to the prevalence and transmission of *P. vivax*, but it is unclear if either relapses or reinfections are more responsible for clinical vivax malaria cases. To assess the contribution of relapses as well as homologous and heterologous re-infections to clinical vivax malaria, a series of experiments using the rhesus macaque - cynomolgi malaria model were conducted. Clinical and parasitological data were collected daily for 100 days during the initial infection and for 45 – 60 days during the re-infections. Relapses did not induce significant clinical alterations, and when minor changes were observed, they resolved without the need for clinical intervention. Homologous reinfections resulted in considerably lower parasite burden and minimal alterations, if any, in clinical parameters, similar to relapses. Interestingly, infection with a heterologous strain of *P. cynomolgi* did result in significant changes in clinical parameters, although there may have been some clinical protection conferred by the initial infections given the evidence of self-controlled acute parasitemia upon heterologous challenge. Collectively, the data from these experiments suggest that relapses caused by *P. vivax* parasites that are genetically similar to parasites in primary infections and homologous re-infections likely do not contribute significantly to clinical vivax malaria cases. Contrastingly, infections with genetically dissimilar strains of *P. vivax* can have pathological consequences, although severity may be less than with the initial infection. Overall, these studies demonstrate that there is much to learn about the clinical consequences of relapses and re-infections and also highlight that the dynamics of *P. vivax* infections are complicated.

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PLASMODIUM FALCIPARUM FIELD ISOLATES TRIGGER APOPTOSIS PREFERENTIALLY IN HUMAN BRAIN ENDOTHELIAL CELLS COMPARED TO PULMONARY ENDOTHELIAL CELLS

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Plasmodium falciparum infection can progress unpredictably to severe forms including respiratory distress and cerebral malaria. The mechanisms underlying the variable natural course of malaria remain elusive. Here we used cocultured brain and pulmonary endothelial cells challenged with *P. falciparum* field isolates taken directly from malaria patients and scrutinized their capacity of inducing endothelial apoptosis via cytoadherence or not. A total of 27 *falciparum falciparum* isolates were collected from patients with uncomplicated malaria (n=25) or severe malaria (n=2). About half the isolates (n=17) were able to bind brain endothelial cells (12 isolates, 44%) or lung endothelial cells (17 isolates, 63%) or both (12 isolates, 44%). Sixteen (59%) of the 27 isolates were apoptogenic for brain and/or lung endothelial cells. The apoptosis stimulus could be cytoadherence, direct cell-cell contact without cytoadherence, or diffusible soluble factors. While some of the apoptogenic isolates used two stimuli (direct contact with or without cytoadherence, plus soluble factors) to induce apoptosis, others used only one. Among the 16 apoptogenic isolates, eight specifically targeted brain endothelial cells, one lung endothelial cells, and seven both. These results suggest that *falciparum falciparum* field isolates killing brain endothelial cells are more prevalent than those killing pulmonary endothelial cells and may provide new insights into host-parasite interactions.

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CHARACTERIZATION OF ANTIBODIES AGAINST *PLASMODIUM FALCIPARUM* INVASION PROTEIN PFMSP10

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The aim of this study is to evaluate monoclonal antibodies (mAb) generated against pfMSP10 (Merozoite surface protein 10) and their functional role to inhibit the invasion of Peruvian *Plasmodium falciparum* isolates using the Growth inhibition Assay (GIA) *in vitro* as well as the potential use of pfMSP10 in malaria diagnostics. Seven mAb (Gen Script and Abmart) and a polyclonal antisera were evaluated by Western Blot (WB) against the recombinant MSP10 full protein (rMSP10). Synchronized and purified schizonts from *P. falciparum* 3D7 and their concentrated supernatant were obtained by ultrafiltration. Detection of pfMSP10 protein was also evaluated in synchronized ring stage of *P. falciparum* cultures from 1 to 12 hours post-invasion. IFA assays were also carried out. In addition a quantitative direct sandwich ELISA for rMSP10 was developed using all the possible combination of the eight antibodies in evaluation. From all the eight evaluated antibodies, only one mAb (anti pfMSP10-1)

and the polyclonal antisera showed a strong reaction band against pfMSP10 and no cross reaction bands against non-infected RBC. Results by WB showed the presence of an approximately 68 kDa band in purified schizonts and rings stages parasites from 1 to 4 hours post invasion and faint band at 12 hours post-invasion, the binding of these antibodies to mature schizonts was corroborated by IFA. Results from the quantitative ELISA showed that three antibodies combinations were able to detect concentrations from 10,000 - 312.5 pg/ml of rMSP10 (OD 2.0 - 0.5 DS 0.04 R2, 0.92) within a standard curve. Quantification of pfMSP10 will be performed using patient serum and culture samples. GIA *in vitro* assays are underway using Peruvian *P. falciparum* isolates in order to evaluate whether the mAb against MSP10 is capable of binding to erythrocytes to inhibit the invasion of merozoites into erythrocytes.

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CHARACTERIZATION OF A NOVEL ERYTHROCYTE BINDING PROTEIN OF *PLASMODIUM VIVAX*

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Erythrocyte invasion by malaria parasites is essential for blood stage development. In *Plasmodium vivax* the interaction between the Duffy binding protein (DBP) and its cognate receptor, the Duffy antigen receptor for chemokines (DARC), on human erythrocytes is central to blood-stage infection. Contrary to this established pathway of invasion, recent studies have reported evidence of *P. vivax* transmission among Duffy blood-group negative individuals, suggesting that the parasite might have evolved an alternative pathway to infect this group of individuals. Recently, a second distinct DBP-like erythrocyte binding protein (EBP2) that may be the ligand in an alternate invasion pathway has been discovered in *P. vivax*. This study characterizes this novel ligand and determines its potential role in reticulocyte invasion by *P. vivax* merozoites. Our data demonstrates that EBP2 preferentially binds to young (CD71High) Duffy positive (Fy+) reticulocytes with minimal binding capacity for Duffy negative reticulocytes. EBP2 is antigenically distinct from DBP and can be functionally inhibited by anti-EBP2, but not anti-DBP antibodies. Consequently, our results do not support EBP2 as a ligand for invasion of Duffy negative blood cells, but instead EBP2 may be a ligand for an alternate invasion pathway of Duffy positive reticulocytes.

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PATHWAY GROUP LASSO INTEGRATION OF METABOLOMICS AND TRANSCRIPTOMICS TO CHARACTERIZE MALARIA INFECTION IN RHESUS MACAQUES

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We have conducted a detailed systems biology study of malaria using an infection model of *Plasmodium cynomolgi* in rhesus macaques - a model for *P. vivax* malaria. To this end, we have generated a wide array of biological, clinical and multi-omic data sets on NHPs. Among the various -omic data types, transcriptomics and metabolomics are among the most widely used high-throughput technologies. Integration of these two data types is therefore critical to the advancement of contemporary biomedical science. Here, we report a novel approach of such integration in a framework of group LASSO, and demonstrate its application to understand metabolic changes that occur during malaria infection. For this study, five rhesus macaques were infected with P. *cynomolgi* sporozoites and studied for 100 days post inoculation to enable the study of early infection, acute disease, and relapses. Additionally, five uninfected rhesus were studied with antimalarial treatment only. LASSO is widely used for feature selection and shrinkage estimation, which reduces the variance of regression coefficients. Group information from well-curated pathways was added to these regression models, as a means of incorporating prior knowledge into the analysis. The leading principal components of each pathway group from each omic data type were combined into an integrated matrix that was then used to test for association with clinical measures of malaria illness. Using this method, we identified key differences in metabolism between infected and non-infected rhesus macaques. In both groups, changes in porphyrin metabolism, which is central to heme synthesis, was selected as an important biological pathway. Other pathways that were shared between infected and uninfected primates were glycerophospholipid metabolism and linoleate metabolism. Tyrosine and tryptophan metabolism were significantly changed in infected primates, and not in the uninfected animals. Overall, pathway group LASSO is a novel and effective method of integrating metabolomics and transcriptomics data from large-scale studies, and is a useful tool to provide high-quality mechanistic hypotheses.

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MARKERS OF ANEMIA IN KENYAN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA

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Severe malarial anaemia (SMA) is the most common complication of *Plasmodium falciparum* infections, resulting in mortality rates that may exceed 30% in children (less than 5years) living in holoendemic transmission areas. One strategy for reducing the morbidity and mortality associated with SMA is to identify biomarkers that can be utilized for prompt diagnosis and treatment of the disease. Currently, there are no such reliable comprehensive biomarkers for SMA from the inflammatory, iron, hypoxia, and erythropoietic pathways. As such, we measured anemia markers in Kenyan children (3-36 months, n=278) presenting with acute illness at a rural hospital in Siaya, Kenya. Children were categorized into three groups based on anemia status and any density parasitemia: aparasitemic (n=56); non-SMA (Hb>5.0g/dL, n=168); and SMA (Hb<5.0g/dL, n=54). The following measures were obtained and compared across the groups: C-reactive protein (CRP); total iron; total iron binding capacity (TIBC); ferritin, carbon(IV)oxide; creatinine; bilirubin; total bilirubin; and glucose. The results indicated significantly higher median levels in SMA group compared to the other anemia phenotypes for CRP (130mg/dL, p=0.0001), total iron (89ug/dL, p=0.019), ferritin (200ng/ml, p=0.0001), direct bilirubin (2.5mg/dL, p=0.008) and total bilirubin (2.25mg/dL, p=0.0001), while creatinine levels were significantly lower in anemia groups compared to the aparasitemic controls (0.35mg/dL, p=0.030). Data here suggest that the levels of these markers may be useful predictors of anemia disease severity in *P.falciparum* malaria holoendemic transmission areas.

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CLINICAL AND LABORATORY PROFILE OF COMPLICATED MALARIA IN THE COLOMBIAN PACIFIC COAST

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Complicated malaria remains an important public health problem especially in low-income settings, where access to health services is difficult and most of the fatal outcomes occur. Although several studies have been conducted in Colombia, most of them are retrospective and present inherent limitations. A cross-sectional study was performed in

hospitalized patients with complicated malaria in four low-to-moderate endemic regions on the Colombian Pacific Coast during 2014-2016, to describe the clinical, laboratory, and sociodemographic characteristics of study participants. A total of 169 complicated malaria patients between zero and 82 years old were enrolled, including 27 children ≤5 years old and 16 pregnant women. *Plasmodium falciparum* was the main parasite species (70%), followed by *P. vivax* (27%) and mixed malaria (3%). Most common laboratory complications were severe thrombocytopenia (30%), hepatic failure (28%) and severe anemia (16%). Main clinical complications were oral intolerance (33%) and prostration (17%). Two deaths due *to P. vivax* and *P. falciparum* malaria were registered. Patients with *P. falciparum* had significantly higher creatinine levels (1.0 vs 0.6 mg/dL, p=0.0002) and aminotransferases (AST: 102.5 vs 34.0 IU, p<0.0001, ALT: 104.0 vs 47.5 IU, p=0.0014) levels than *P. vivax* cases. In contrast, *P. vivax* patients presented significantly lower hemoglobin levels than *P. falciparum* cases (8.0 vs 9.9 g/dL, p=0.0109). Patients who presented more than one complication simultaneously (52%) had significantly lower platelet counts and higher bilirubin levels regardless of parasite species, as well as higher parasitemias, AST/ALT, creatinine and BUN levels in *P. falciparum* cases. No bacterial co-infections were found. Quibdó was the region with the highest proportion of complicated malaria cases (57%), with transmission of both *Plasmodium* species. Therefore, the high prevalence of complicated malaria in this region, together with more severe anemia in *P. vivax* and worse renal and hepatic function in *P. falciparum* infections, demands particular attention to prevent the higher morbidity and potential mortality.

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PLASMA HAPTOGLOBIN AS A MARKER OF CLINICAL SEVERITY IN GAMBIAN AND MALAWIAN CHILDREN INFECTED WITH *PLASMODIUM FALCIPARUM*

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Elevated cell-free hemoglobin (Hb) is associated with disease severity in adults infected with *Plasmodium falciparum*. However, cell-free Hb can be elevated not only by intravascular hemolysis, but also by hemolysis induced by blood drawing/sample processing. Plasma haptoglobin (Hp), an endogenous Hb scavenging protein, binds cell-free Hb and the Hp-Hb complex is removed from circulation via CD163 on reticuloendothelial cells. Thus Hp falls during intravascular hemolysis, but is minimally affected by *in vitro* red cell lysis. We measured Hp concentration in plasma obtained from children presenting with uncomplicated or severe malaria, as well as healthy children of similar age, using an ELISA (Alpco). Clinical severity was defined per WHO criteria as uncomplicated (UM) or severe malaria (SM). In The Gambia, plasma Hp concentration was 44.5 [15.6-79.9], 4.8 [2.4-50.2], and 2.6 [2.4-5.5] mg/dL among healthy children and children with UM or SM, respectively. ROC curve analysis revealed that a Hp threshold of 24.0 mg/dL distinguished UM from healthy children (AUC 0.68 [0.57-0.79], sens 0.66, spec 0.72), and a threshold of 9.2 mg/dL distinguished SM from healthy children (AUC 0.79 [0.70-0.89], sens 0.77, spec 0.78). A Hp threshold of 4.4 g/dL distinguished UM from SM poorly (AUC 0.60 [0.49-0.70] sens 0.58, spec 0.61). In Malawi, plasma Hp concentration was 177.2 [37.4-330.4], 1.3 [0.5-249.1], and 0.5 [0.01 - 2.4] mg/dL among healthy children, and children with UM or SM, respectively. ROC curve analysis revealed that a Hp threshold of 18.1 mg/dL distinguished UM from healthy children (AUC 0.64 [0.50-0.80], sens 0.57, spec 0.83) and a threshold of 10.6 mg/dL distinguished SM from healthy children (AUC 0.82 [0.71-0.92], sens 0.86, spec 0.77). A threshold of 5.5 mg/dL distinguished UM from SM poorly (AUC 0.63 [0.50-0.77], sens 0.53, spec 0.75). These data reveal that Hp depletion, an indicator of massive intravascular hemolysis, is prevalent not only among patients with severe malaria, but also among patients with uncomplicated malaria. Thus massive hemolysis might be necessary, but it is not sufficient to cause the clinical syndrome of severe malaria.

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CELL-SPECIFIC DELETION OF TISSUE FACTOR ALTERS THE IMPACT OF *PLASMODIUM CHABAUDI CHABAUDI* AS INFECTION ON MURINE PREGNANCY OUTCOME

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Sequestration of *Plasmodium falciparum*-infected erythrocytes in the maternal blood space of the placenta results in a severe clinical manifestations of this disease, placental malaria (PM). PM results in disruption of placental function, leading to low birth weight or fetal loss. Recent evidence of a procoagulant state in PM, including extensive fibrin deposition and Tissue Factor (TF) expression in affected tissues, indicates dysregulated coagulation contributes to malaria pathogenesis, but the molecular basis for these pathologies remains incompletely understood. Timed pregnancy experiments were conducted using female mice with floxed TF expressing Cre-recombinase under the Tie2 promoter (Tie2Cre+) or under the Lysozyme M promoter (LysMCre+) and their phenotypically normal Cre-negative littermates (Tie2Cre- and LysMCre-, respectively). Upon observation of a vaginal plug (gestation day (GD) 0), mice were infected with 1000 *Plasmodium chabaudi* AS-infected erythrocytes. Embryo viability and health were assessed at sacrifice at GD12, and placental pathology was assessed in hematoxylin and eosin-stained histological sections and indirect immunolocalization of TF and fibrin were performed in unstained sections. Malaria-infected, pregnant (IP) Tie2Cre+ mice exhibit improved embryo health and significantly increased pregnancy-associated weight gain (p=0.0338) at GD12 relative to IP Tie2Cre- littermates. Though there is no significant difference in the magnitude of peak percent weight gain or parasitemia between the two strains, IP LysMCre+ mice abort and reach peak parasitemia two days earlier than IP LysMCre- mice. These results indicate that TF on both myeloid cells and either maternal endothelium or fetal-derived trophoblast play a significant role in determining pregnancy outcome during malaria infection. Future experiments seek to understand the mechanisms by which TF on hematopoietic cells may be influencing pregnancy outcome, particularly in how it affects platelet activation and aggregation, and the source of TF causing the phenotype seen in infected Tie2Cre+ mice.

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ANTI-OXIDANT THERAPY SLIGHTLY IMPROVES PREGNANCY OUTCOME IN A MOUSE MODEL OF PLACENTAL MALARIA

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Placental malaria, characterized by sequestration of *Plasmodium falciparum* in the maternal placental blood space and associated inflammatory damage, contributes to poor birth outcomes and ~200,000 infant deaths annually. Specific mechanisms that contribute to placental damage and dysfunction are not completely understood. To assess a potential role of oxidative stress, a marker for lipid peroxidation, 4-hydroxynonenal was quantified by immunohistochemistry in placentas of C57BL/6 mice infected in early pregnancy with *P. chabaudi* AS and malaria-infected Kenyan women. Widespread evidence of lipid peroxidation was observed and was associated with higher anti-oxidant gene expression in

conceptuses of infected mice. To assess the extent to which this oxidative damage and response might contribute to poor birth outcomes and be amenable to therapeutic intervention, infected pregnant mice were injected with N-acetylcysteine (NAC), a free radical scavenger, or tempol, an intracellular superoxide dismutase mimetic, or were given tempol in drinking water. Mice treated with NAC experienced pregnancy loss at the same rate as control animals; in contrast, tempol-treated mice exhibited subtle improvement in embryo survival at gestation day 12. However, immunohistochemical staining for 4-hydroxynonenal was not consistently reduced in placentas of tempol-treated mice. Thus, while oxidative stress is remarkable in placental malaria and its mitigation by anti-oxidant therapy may improve pregnancy outcomes, additional more effective interventions must be tested.

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TEMPERATURE INDUCED CHANGES IN GLOBAL GENE EXPRESSION PROFILES OF MALARIA-CARRYING MOSQUITOES

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By regulating the abundance and distribution of the mosquito vector, environmental factors such as temperature may play a major role in shaping the worldwide incidence of the scourge that is malaria. In general, the relationship is non-linear and indicates distinct temperature optima within which transmission is maintained. Although its effect on vectorial capacity is well appreciated, the patterns and processes underlying the observed phenotypes remain largely obscure. Additionally, its effect on next-generation vector control measures such as transgenic mosquitoes, is even less characterized, but is likely to play a major role in their efficacy. In the current study, we employed RNA-sequencing to investigate global gene expression profiles in the midguts, salivary glands and carcasses of wild-type and transgenic *Anopheles* stephensi mosquitoes infected with *Plasmodium falciparum* at temperatures of 20°C, 27°C and 32°C. Preliminary analyses suggest large scale changes in gene expression in response to temperature, with distinct enrichment in specific pathways. Targeted knockdown of a specific set of genes with RNA interference for instance, will help elucidate their contribution to vectorial competence as well as predict their effects in the face of a variable environment.

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EXPERIMENTAL DESIGN OF *PLASMODIUM KNOWLESI* INFECTION IN SUSCEPTIBLE VERSUS REFRACTORY NON-HUMAN PRIMATE MODEL HOSTS

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The Malaria Host-Pathogen Interaction Center (MaHPIC) and the Host Acute Models of Malaria to study Experimental Resilience (HAMMER) are a large systems biology consortia investigating multi-'omic approaches to study *Plasmodium* species host-pathogen interactions in non-human primate (NHP) hosts, to model human malaria infections. Longitudinal studies with *P. knowlesi* in Macaca mulatta (Rhesus monkey) and *M. fascicularis* (Kra monkey) were designed to identify features at the host-pathogen interface conferring varying degrees of host susceptibility to parasite infection. We expect the susceptible Rhesus (n=8) and refractory Kra monkey (n=8) to manifest different disease profiles and severity at the host-pathogen interface following inoculation with *P. knowlesi* sporozoites, identifying targets unique to disease response. Parasite loads and stages are assessed daily alongside clinical parameters and continuous internal telemetry during the course of infection timeline to anticipate significant points of infection observed during the disease progression. Extensive samples are recovered at each time point to monitor the dynamic changes in the host immune status, erythrocyte phenotype, gene expression and metabolic state by means of immune profiling implementation, transcriptomics, proteomics, lipidomics, microbiome, metabolomics and tissue analysis. To further our understanding of pathological significance during the course of infection, necropsies were performed at various points of infection allowing deeper analysis of affected tissues and organ systems uniquely influenced between these NHP cohorts. Through this set of experiments, we aim to identify host features that confer protection against malaria disease and relate these observations to developments intended to reduce host susceptibility to *Plasmodium* infections.

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POPULATION PHARMACOKINETICS AND PHARMACODYNAMICS OF LUMEFANTRINE IN YOUNG UGANDAN CHILDREN TREATED IN COMBINATION WITH ARTEMETHER FOR UNCOMPLICATED MALARIA

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Artemether-lumefantrine is the most widely recommended first-line treatment for malaria. The objective of this study was to describe the population pharmacokinetics and pharmacodynamics of lumefantrine when used in the combination therapy, artemether-lumefantrine, in Ugandan children 6 months to 2 years of age. Capillary whole blood samples were collected over 21 days in 105 children treated for 249 episodes of uncomplicated *Plasmodium falciparum* malaria with standard fixed-dose artemether-lumefantrine (twice daily x 6). Population pharmacokinetic analysis of evaluable lumefantrine concentrations (n=806 in 101 children) employed a 2-compartment open model with first-order absorption. Age was found to have a significantly positive correlation with bioavailability in a model that included allometric scaling. Cox proportional multivariate hazards regression was used to explore the relationship between exposure and clinical outcome. A significant interaction between trimethoprim-sulfamethoxazole prophylaxis and a day 7 lumefantrine concentration threshold of 200 ng/mL was present. Children not on trimethoprim-sulfamethoxazole with lumefantrine concentrations below 200 ng/mL had a 3-fold higher hazard of 28-day recurrent parasitemia compared to those with concentrations above 200 ng/mL (p=0.0007). In contrast, the 28-day risk of recurrent parasitemia was not significantly different in children on trimethoprim-sulfamethoxazole based on a lumefantrine threshold of 200 ng/mL (p=0.10). Lumefantrine concentration on day 3 was a stronger predictor of 28-day recurrence compared to day 7 levels. Our data demonstrate that age is a significant determinant of lumefantrine bioavailability, and in the absence of TS, lumefantrine exposure is a determinant of 28-day recurrent parasitemia in this age group. Further refinement of artemether-lumefantrine dosing guidelines in young children may be warranted.

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SURVEILLANCE FOR SULFADOXINE-PYRIMETHAMINE (SP) RESISTANT MALARIA PARASITES IN THE LAKE AND SOUTHERN ZONES, TANZANIA USING POOLING AND NEXT-GENERATION SEQUENCING

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Malaria in pregnancy (MiP) remains a major public health challenge in areas of high malaria transmission; intermittent preventive treatment in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP) is recommended to prevent the adverse consequences of MiP. The effectiveness of IPTp-SP is maintained despite high prevalence of quintuple-mutant haplotypes associated with SP resistance (estimated by the prevalence of mutation at dhps540). The effectiveness of SP for IPTp may be reduced in areas where the dhps581 mutation is found in conjunction with the quintuple mutant background. The dhps581 mutation is common in the Tanga Region of northern Tanzania, but there are limited data from other areas. We investigated the prevalence of molecular markers of SP resistance in malaria parasites in the Lake and Southern Zones of Tanzania. A cross-sectional survey was conducted in 14 health facilities (HF) in seven regions of mainland Tanzania from April to June, 2015. A total of 1,750 dried blood spot (DBS) samples were collected (117 to 160 samples per facility) from consenting patients presenting to the outpatient department who had positive rapid diagnostic tests for malaria. Patients with recent exposure to SP or related drugs were excluded. DNA was extracted from the DBS, pooled by HF, and analyzed by Illumina MiSeq deep sequencing to yield estimates of mutated parasite allele prevalence at each locus of interest. The dhps540 mutation was prevalent across all 14 sites, ranging from 55% to 98.4%, with higher prevalence in Lake Zone compared to Southern Zone. Prevalence of the dhps581 mutation ranged from 0 to 2.4%, with the exception of Kayanga HF (Kagera Region, Lake Zone) where 24.9% of sequences were mutated. The dhfr164 mutation was detected only at Kanyanga HF (0.06%). Although the quintuple mutant was highly prevalent, dhps581 remains geographically restricted, suggesting that IPTp-SP may remain effective in most of Tanzania. However, additional surveillance, particularly in and around Tanga Region is warranted. In addition, a better understanding of the effect of the dhps581 mutant on the efficacy of IPTp-SP is needed.

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UPDATE ON THE ASEXUAL AND SEXUAL STAGE-EFFICACY OF ATOVAQUONE-PROGUANIL AND SINGLE LOW DOSE PRIMAQUINE WITH OR WITHOUT ARTESUNATE IN CAMBODIA

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Atovaquone-proguanil (AP) is a safe, well-tolerated drug for resistant Plasmodium falciparum. However, concerns over rapid development of blood-stage resistance and cost have limited its use to high risk areas in Southeast Asia. While combining AP with artesunate (AS) was previously shown to improve efficacy against MDR P. falciparum in Thailand in 2004, we evaluated current efficacy in Cambodia given recent failures of first-line therapies and assessed the effect of AP use with PQ on gametocyte carriage. Subjects were randomized 1:1 to an open label fixed-dose 3 day AP regimen with or without 3 days of co-administered artesunate (ASAP). Subjects were administered single low dose primaquine (15mg) on day 1 to prevent gametocytemia. A total of 205 subjects with P. falciparum or mixed P. vivax infection were enrolled from December 2014-October 2015 at two sites, Anlong Veng (AV; n=157) and Kratie (KT; n=48) Cambodia. Subjects were followed for 42 days for malaria recurrence and gametocyte carriage. PCR-adjusted ACPR at 42 days was similar for the two regimens - 93% (95% CI = 86-98) for AP vs. 95% (95% CI = 88-98) for ASAP (p=0.73). However, of 17 total P.f. recurrences, 16 (9%; 13 confirmed recrudescences) occurred at the AV site compared with only 1 (3%) in KT. P. falciparum remained sensitive to atovoquone in-vitro, with mean pretreatment IC50 4.76 (95% CI=5.3-8.3) in AV and 3.21 (95% CI=2.9-6.7) in KT (p=0.0096). Median parasite clearance time (PCT) was shortest in KT at 56 hrs (p <0.001) in ASAP-treated subjects and 68 hrs for AP, compared to 72 hrs for both treatment arms in AV. On day 2 post PQ, gametocyte carriage was lower in the ASAP (17%) vs. AP (29%) treatment arm and reached statistical significance by day 3 (11% in ASAP vs. 29% in AP, p=0.0012). Drug level analysis and DNA sequencing analysis for cytb mutations are in progress at the time of submission. While atovaquone-proguanil remains effective in Cambodia both clinically and in vitro, co-administration of AS with PQ in the ASAP arm might have contributed to reduced gametocyte carriage, with potential implications on how AP should be deployed in Cambodia.

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HEMOGLOBIN E RED BLOOD CELLS DO NOT INFLUENCE THE ANTIPLASMODIAL ACTIVITY OF ARTEMISININ *IN VITRO*

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Artemisinin (ART)-resistant Plasmodium falciparum malaria, defined as a parasite clearance half-life >5 h following treatment, is widespread in Cambodia, where hemoglobin E (HbE) is highly prevalent. HbE red blood

cells (RBCs) have excess α-globin chains and Hb degradation products, causing increased oxidative stress. The endoperoxide moiety of ARTs exerts antiplasmodial activity within host RBCs via oxidation reactions, suggesting that interaction effects involving parasites, RBCs, and ARTs may occur. In a clinical study of ART-resistant malaria in Cambodia, parasite clearance half-life was nonsignificantly increased in HbE patients (comprising 43% of the total) compared to HbA patients, but this difference was not significant (0.55 h, P=0.078). Nonetheless, we hypothesized that HbE diminishes the antimalarial activity of dihydroartemisinin (DHA, the active metabolite of ARTs). To explore this, we compared the antiplasmodial activity of DHA in HbE and HbA RBCs using: (i) ART-sensitive/K13-wildtype and ART-resistant/K13-mutant parasites from Cambodia; (ii) the in-vitro ring-stage survival assay (RSA), where higher % survival values associate with longer parasite clearance half-lives; and (iii) HbAA, HbAE, and HbEE RBCs that lack α-thalassemia and G6PD-deficiency genotypes. In all three RBC types, % survival values: were <1% for ART-sensitive parasites and 7-70% for ART-resistant parasites; increased according to K13 mutation (Y493H<C580Y<R539T); and were not significantly different (Kruskal-Wallis test, P>0.05). To test whether senescent, oxidatively-stressed RBCs affect % survival values in the RSA, we used Percoll to fractionate HbAA RBCs into young and old cells. In these cell types, % survival values for ART-sensitive and ART-resistant parasites were also not significantly different. These data indicate that neither HbE nor intraerythrocytic redox status influence the in-vitro antiplasmodial effect of a pharmacologically-relevant dose of DHA, suggesting these factors do not prolong the parasite clearance half-life in patients treated with ART.

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ASSESSING THE IMPACT OF MALNUTRITION ON THE TREATMENT OUTCOME OF ARTEMISININ-BASED COMBINATION THERAPY IN UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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In children under 5 years of age little is known about the effect of malnutrition on the outcome of *Plasmodium falciparum* (Pf) malaria treatment with an Artemisinin-based Combination Therapy (ACT). Contrasting reports may reflect heterogeneity in the study population, diversity in transmission intensity, use of different growth metrics or small sample sizes. A systematic search of the WWARN data repository and online literature databases identified 35 Pf efficacy studies in which weight and height were both recorded in children under 5 years of age treated with artemether-lumefantrine (AL), artesunate-amodiaquine (ASAQ), dihydroartemisinin-piperaquine (DP) or artesunate-mefloquine (ASMQ). Only studies with at least 28 days of follow-up were included in the analysis. Four anthropometric indicators were reviewed: weight-for-height, height-for-age, weight-for-age (calculated using WHO igrowup tool), and the mid-upper arm circumference. An a priori data analysis plan was developed to investigate the association between anthropometric indicators and antimalarial efficacy. Individual patient data were collated from 11,528 children (99% from Africa), treated with AL (44%), ASAQ (27%), DP (23%) or ASMQ (5%). 298 recrudescences and 1,792 reinfections confirmed by PCR were recorded. The overall risk of failure (i.e. recrudescence) was greatest in children with wasting (weight-for-height (whz)<-1). After adjusting for ACT regimen, dose administered and initial parasite density, the treatment failure risk by day 42 was greater in children 1-3 years of age compared to other children (HR=1.50 [95%CI 1.15-1.96]; p=0.0030) and in children with wasting compared to those without wasting (HR=1.41 [95%CI 1.07-1.86]; p=0.013). More severe wasting (whz<-2) was associated with an increased risk of reinfection compared to children without wasting (HR=1.26 [95%CI 1.09-1.45]; p=0.002). This pooled analysis highlights the risk of ACT treatment failure and reinfection in children with wasting, especially in those aged 1-3 years. The consequences on mortality in children suffering from acute global malnutrition warrants further investigation.

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IMPACT OF DIFFERENT MALARIA CHEMOPREVENTION REGIMENS FOR PREGNANT UGANDAN WOMEN ON *PLASMODIUM FALCIPARUM* DRUG RESISTANCE-MEDIATING POLYMORPHISMS

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In a recent randomized trial comparing intermittent preventive therapy regimens to prevent malaria in 300 pregnant women in Tororo, Uganda, dihydroartemisinin-piperaquine given once a month (DPqm) or every 2 months (DPq2m) was superior to sulfadoxine-pyrimethamine given every 2 months (SPq2m). For this study we analyzed the impacts of the different chemoprevention regimens on *Plasmodium falciparum* genetic polymorphisms that affect sensitivity to a number of antimalarial drugs. Blood samples collected monthly from asymptomatic women were analyzed for *P. falciparum* DNA with a highly sensitive loop mediated isothermal amplification (LAMP) assay. All 635 samples positive by LAMP plus samples from all 75 episodes of symptomatic falciparum malaria are now undergoing characterization of polymorphisms in relevant genes encoding putative drug transporters (pfcrt and pfmdr1) and folate enzymes (pfdhfr and pfdhps). We report preliminary data for transporter polymorphisms. The prevalence of mutations at each studied allele (pfcrt K76T and pfmdr1 N86Y, Y184F, and D1246Y) was the same in each arm of the trial in parasites identified before initiation of study drugs. In parasites identified after initiation of study drugs, for pfmdr1 N86Y, the prevalence of a mixed or mutant genotype was higher in the DPqm (91.7%, p<0.001) and DPq2m (50.0%, p=0.001) arms compared to the SPq2m arm (24.9%). For pfmdr1 Y184F, the prevalence of a mixed or mutant genotype was higher in the DPqm (96.2%, p<0.001) and DPq2m (88.6%, p=0.001) arms compared to the SPq2m arm (61.4%). Inconsistent or non-significant trends were seen for the two other studied polymorphisms. Analyses of polymorphisms in pfdhfr and pfdhps are ongoing. Of note, monthly DP strongly selected for the pfmdr1 86Y mutation in subsequent infections. This mutation appears to be associated with decreased activity of AQ, but increased activity of lumefantrine, suggesting that chemoprevention with DP might optimally be utilized when artemether-lumefantrine is the first line drug to treat malaria, so that selection of resistance to partner drugs is minimized.

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DRUG RESISTANCE AND RELAPSE IN CAMBODIAN *PLASMODIUM VIVAX*

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Assessment of antimalarial drug resistance is complicated in *Plasmodium vivax* by the lack of *in vitro* culture system. Most studies of drug resistance in *P. vivax* therefore rely on patient data and the observation of parasites in blood after treatment. However, such studies are complicated by the difficulty to differentiate resistant parasites from re-infections and relapses. To overcome these limitations, we studied *P. vivax*-infected patients treated with chloroquine (CQ) and followed for 80 days in an area without malaria transmission (to exclude reinfections). We tested each patient blood for *P. vivax* DNA by PCR every 8 hours until the parasites were not detectable anymore and then every second day for the rest of the study. Our analyses showed that, for all patients, parasite DNA was not detectable 5 days after drug treatment suggesting that there is little CQ resistance in this population. However, more than half of the patients show re-occurences of *P. vivax* parasites during the 80-day monitoring period suggesting that

relapses occur frequently. Interestingly, none of the relapses occurred when the CQ concentration was above therapeutic level, and the few relapses leading to clinical malaria were successfully cleared by CQ treatment, confirming that these parasites are susceptible to CQ. To further analyze the susceptibility of the parasites to CQ, we genotyped at 100 SNPs parasite DNA extracted from the patient blood samples collected before, 8 hours and 16 hours after treatment and determined, for each infection, the relative susceptibility of each clone to CQ. We also used genotyping and whole genome sequencing to characterize the parasites in the primary infections and relapses to confirm, for a subset of the patients, that the relapses were caused by a parasite that is not detected in the primary infection. Overall, our study did not reveal any CQ resistance among Cambodian *P. vivax* but suggests that pervasive relapses might have confounded previous assessments of drug resistance in patient studies.

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BEHAVIOR OF *PLASMODIUM FALCIPARUM* AGAINST ARTEMISININ-BASED COMBINED THERAPY FOR MALARIA: EVALUATION OF *EX VIVO* SENSITIVITY AND PARASITE DORMANCY

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Approximately 3.3 billion people live at risk of contracting malaria worldwide, with 198 million cases and 584,000 deaths in 2013. There are evidences of decreased efficacy of artemisinin and its derivatives in isolates of *Plasmodium falciparum* in some countries. The dormancy in *P. falciparum* has been proposed as a mechanism of tolerance to artemisinin. In this study, blood samples from patients infected in African and Caribean countries were processed in order to perform *ex vivo* sensitivity assays and *in vitro* dormancy evaluation. For *ex vivo* sensitivity we tested dihydroartemisinin (4-1,000nM), artesunate (0.1-100nM), lumefantrine (3.1-200nM) and mefloquine (0.2-1,000nM). Parasites were incubated in RPMI 1640 with a haematocrit of 5% and parasitemia of 1%. After 96 hours thick blood films were prepared and the number of schizonts/200 parasites was counted. Blood from wells with only rings was transfered to a new microplate for monitoring the dormancy phenomenon for a period of 41 days. Flow cytometry using 1,2,3-Rhodamine and DAPI was performed to assess the viability of parasites. As far as the *ex vivo* sensitivity assays is concerned, minimal inhibitory concentrations ranged from 10.2 to 250nM for dihydroartemisinin, from 50 to 200nM for lumefantrine, from 3.7 to >100nM to artesunate and from 62.5 to 250nM for mefloquine. In the dormancy assays with clinical and reference samples, schizonts were observed after pressure with 62.5, 250 e 1,000nM of dihydroartemisinin. The recovery period of parasites ranged from 4 to 40 days. For lumefantrine, schizonts have emerged only in the reference isolate in days 7 and 12 after exposition to 66.6nM and 200nM respectively. It is worrying to note that parasite growth inhibition was only achieved in high concentrations of dihydroartemisinin and lumefantrine, used worldwide for malaria treatment. As the flow cytometry showed viability of ring stages after drug pressure, our results suggest that the assays based on microscopy could underestimate the response of *P. falciparum* to artemisinin-based combined therapy. To our knowledge, the dormancy phenomenon has never been reported before for lumefantrine.

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ELUCIDATING THE MECHANISM OF PIPERAQUINE RESISTANCE IN *PLASMODIUM FALCIPARUM* MALARIA IN CAMBODIA

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Artemisinin combination therapies (ACTs) are currently the first-line treatments for *Plasmodium falciparum* malaria worldwide. ACTs, which combine a short-acting artemisinin derivative with a long-acting antimalarial partner drug with a different mechanism of action, are designed to efficiently clear parasitemia and prevent the development of drug resistance. In some countries of Southeast Asia (SEA), the current treatment for *P. falciparum* malaria is the ACT dihydroartemisinin (DHA)-piperaquine (PPQ). Unfortunately, the emergence and spread of DHA-PPQ resistance has now been reported at multiple sites in Western Cambodia, which poses a severe risk of widespread resistance to DHA-PPQ and other ACTs. Recent fieldwork by our group identified *P. falciparum* strains that show markedly reduced susceptibility to PPQ in Cambodia. In a genome-wide association study of parasite responses to PPQ exposure *in vitro*, we discovered a single-nucleotide polymorphism (SNP) on chromosome 13 coding for an exonuclease that strongly associates with reduced PPQ susceptibility *in vitro* and DHA-PPQ failures in patients. We are currently using the CRISPR-Cas9 system to edit the wild-type and mutant exonuclease SNP into PPQ-resistant and PPQ-sensitive parasites, respectively. After transfections and successful editing events are verified, we will perform PPQ survival assays to determine whether the mutant SNP confers PPQ resistance. We are also genotyping the exonuclease SNP in contemporary parasite isolates from Cambodia and neighboring countries to monitor the spread of PPQ resistance in SEA. In addition to validating the exonuclease SNP as a molecular marker of PPQ resistance, we aim to identify the causal genetic determinant of PPQ resistance and use appropriate biochemical methods to elucidate its molecular mechanism. These studies will provide novel insights into the mechanism of PPQ resistance in *P. falciparum* and will help to monitor and prevent the further spread of PPQ resistance and DHA-PPQ failures in SEA.

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IMPACT EVALUATION AFTER THREE YEARS OF SEASONAL MALARIA CHEMOPREVENTION IMPLEMENTATION BY MASS CAMPAIGNS IN SOUTHERN SENEGAL

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Seasonal Malaria Chemoprevention (SMC) was piloted in 2013 and extended in Senegal from 2014, in the southern regions where malaria transmission is seasonal and intense with 620,000 children under 10 years eligible. It is however important that scaling up of SMC by national Malaria Control Programs is evaluated to document its coverage, the safety profile, the impact on malaria morbidity and the prevalence of molecular markers of drug resistance. In order to monitor malaria morbidity surveillance, 230 health structures were listed by district, with their catchment populations of 2 million inhabitants based on the 2012 census. A sample of 32 health posts and 16 districts hospitals were selected with probability proportional per size (PPS) using systematic random sampling, with respect to geographical coverage and malaria incidence rates to ensure the sample was representative. One month after the third SMC cycle, a

cross sectional survey targeting 2000 children under 10 years of age in 45 villages selected by PPS were recruited to document SMC coverage and drug resistance markers in 2014 and 2015. Overall 3968 mild adverse events were detected during SMC campaigns including 1026 by the national passive system and 2942 by 2 strategies to strengthen the existing system; and 3 serious adverse events (2 anaphylactic shocks and 1 extra pyramidal syndrome) after the administration of almost 2 million SMC treatments. Ninety eighteen percent of children under 10 years received at least one dose of SMC. The coverage for a full treatment course was 74% for the last cycle. Malaria morbidity surveillance showed in 2014 during the months when SMC was administered, a 66% (95%CI 57%,73%) reduction in outpatient malaria cases and a similar reduction in cases of severe malaria. In 2015, malaria incidence has increased in the targeted group due to several factors including changes in diagnosis flowchart with HRP2 rapid diagnostic test and an exceptional rainy season in 2015 showing the importance of continuous monitoring and evaluation programmes of SMC implementation in the Sahelian region.

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ARTESUNATE TO TREAT SEVERE MALARIA IN TRAVELERS: REVIEW OF EFFICACY AND SAFETY AND PRACTICAL IMPLICATIONS

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Artesunate is recommended by the WHO for the first-line treatment of severe malaria worldwide, in adults and children. However, despite solid evidence that artesunate is safe and more effective than quinine in endemic areas, its deployment in non-endemic areas has been slow, due in part to the absence of a Good Manufacturing Practice (GMP) qualification. Using the Prisma method for bibliographic reports we have analyzed published studies (12 retrospectives 1 prospective) and 7 case reports to assess the safety and efficacy of artesunate in travelers with imported severe malaria. Of 574 patients with reported outcome, 22 died (3.83%). No death was attributed to artesunate toxicity. Relatively few side effects were reported including: neurological syndrome (6 cases), temporary deterioration in renal function (3), cutaneous (3) and cardiac (4) manifestations, high blood pressure (1), elevation of liver enzymes (58) and hyperkaliemia (1). A new side effect of artesunate has been uncovered in travelers: Post-Artesunate Delayed Hemolysis (PADH), defined by delayed hemolytic episodes occurring 7 to 30 days after treatment initiation. PADH occurs in 15% of the treated patients. No death or sequelae were reported but blood transfusion was administered in 45% of patients. Weekly follow-up of hematological parameters during one month post-treatment is now recommended. Our analysis confirms the high efficacy and reasonable safety of artesunate in travelers with severe malaria thereby urging for a wider use in non-endemic countries. GMP qualification for the drug and rapid approval by drug agencies is warranted, backed by clear recommendations for optimal use.

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TRENDS OF HIGH REDUCTION OF MALARIA CASES IN SEDHIOU DISTRICT FOLLOWING SEASONAL MALARIA CHEMOPREVENTION FIRST CAMPAIGN: LESSONS LEARNED

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Following research on efficacy and feasibility, Seasonal Malaria Chemoprevention (SMC) was adopted and scaled up in eligible areas in Senegal. Mass campaigns were launched in 2014 in Sedhiou region and targeted children aged more than 2 months to less than 10 years. The objective of this study was to assess effects of SMC following SMC first mass campaign, launched in August 2014. The study was developed in Sedhiou district, South Senegal. Malaria incidence is still high in the region Most of control strategies are ongoing for a total population of 181,594 inhabitants. Data were collected from January 2013 to December 2014 in all health units and in community units through smart phones with GPS. Patients were localized and malaria confirmation were done by Rapid Diagnostic Test. During these two years, 72,570 patients were visited, 40,548 in 2013 and 32,022 in 2014. Overall 5,055 were malaria positive during these two years, with a 74% total reduction in 2014. There was disparities in malaria morbidity reduction; this was 87% among children more than two months to five years, 80% among those six to ten years, 73% among 11 to 15 years and 65% reduction for patients more than 15 years- 20 years; 70% reduction for more than 20 years. Disparities were also observed among health posts and villages where the reduction varied from 22% to 97%. SMC has been highly effective following first year of implementation in Sedhiou district. Major reduction of malaria cases happened among children under 10, but longer integrated evaluation is needed, especially in epidemiology trends, drugs resistance, mid term acceptability and impact of other interventions like universal coverage of Long Lasting Impregnated Nets.

1498

MONITORING THE RESPONSES OF *PLASMODIUM FALCIPARUM* TO ANTIMALARIAL DRUGS USING THE DAPI *EX VIVO* TEST: HIGH FREQUENCY OF *P. FALCIPARUM* ISOLATES RESISTANT TO PYRIMETHAMINE IN DIORO, MALI

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The greatest threat to malaria control is the development of resistance to the antimalarial drugs used to treat uncomplicated and severe Plasmodium malaria in human subjects. In order to monitor the response of *Plasmodium falciparum* to the ACTs in current use we performed an *in vivo* study in which blood samples for parasite isolates were obtained from 31 volunteers with uncomplicated malaria who were 2-15 years of age in the rural commune of Dioro within the Segou Region of Mali. To examine the responses of *P. falciparum* in that region to antimalarial drugs, we performed DAPI *Ex vivo* tests for the isolates from the 31 subjects enrolled in that study from 2014 to 2016. The 8 drugs tested in this study were: Chloroquine (CQ), Piperaquine (PIP), Amodiaquine (AMQ), Lumefantrine (LUM), Mefloquine (MEF), Quinine (QN), Artesunate (ARS), Dihydroartemisinin (DHA), Artemether (ATM) and Pyrimethamine (PYR). Among these 8 drugs, the greatest frequency of resistance based

on the IC50 was found with PYR (71% of isolates with an IC50 > 2000 nM), followed by MEF (35.5% with an IC50 > 30 nM), LUM (22.6% with an IC50 > 150 nM), CQ (22.6% with an IC50 > 100 nM) and DHA (16.1% with an IC50 > 12 nM). In contrast, only 6.5% of the *P. falciparum* isolates had an IC50 > 60 nM for AMQ, an IC50 > 800 nM for QN or an IC50 > 30 nM for ATM. The 71% frequency of *P. falciparum* isolates with Pyrimethamine IC50s > 2000 nM indicates this resistance is common in Dioro and suggests that PYR resistance may limit the efficacy of IPTp for pregnant women because IPTp is based on preventive treatment with SP during pregnancy. In addition, the 22.5% frequency of isolates resistant to LUM poses a threat to ACTs with LUM as the partner drug and potentially increases the risk of late recurrences after the initial parasite clearance due to the artemisinins.

1499

COMBINATORIAL GENETIC MODELING OF PFCRT-MEDIATED DRUG RESISTANCE EVOLUTION IN *PLASMODIUM FALCIPARUM*

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The emergence and spread of drug resistance poses an ongoing threat to the effective treatment and control of *Plasmodium falciparum* malaria. A critical parasite determinant is PfCRT, the primary mediator of CQ resistance (CQR) and a pleiotropic modulator of susceptibility to first-line artemisinin-based combination therapy (ACT) partner drugs. Aside from the validated CQR molecular marker K76T, *P. falciparum* parasites have acquired at least three additional pfcrt mutations, whose contributions to resistance and fitness have remained elusive. Focusing on the quadruple-mutant Ecuadorian PfCRT haplotype Ecu1110 (K76T/A220S/N326D/I356L), we genetically modified the pfcrt locus of isogenic, asexual blood stage *P. falciparum* parasites using zinc-finger nucleases (ZFNs), producing all possible combinations of intermediate pfcrt alleles. Our analysis included the related quintuple-mutant PfCRT haplotype 7G8 (Ecu1110+C72S) that is widespread throughout South America and the Western Pacific. Drug susceptibilities and *in vitro* growth profiles of our combinatorial pfcrt¬-modified parasites were used to simulate the mutational trajectories accessible to parasites as they evolved CQR. Our results uncover unique contributions to parasite drug resistance and growth for mutations beyond K76T and predict critical roles for the CQ metabolite monodesethyl-chloroquine and the related quinoline-type drug amodiaquine in driving mutant pfcrt evolution. Modeling outputs further highlight the influence of parasite proliferation rates alongside gains in drug resistance in dictating successful trajectories. Our findings suggest that *P. falciparum* parasites have navigated constrained pfcrt adaptive landscapes by means of probabilistically rare mutational bursts that led to the infrequent emergence of pfcrt alleles in the field. We recently extended this in an analysis of pfcrt resistance alleles that distinguish the evolution of CQR in Asia and Africa.

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COMPARISON OF HIGH RESOLUTION MELT (HRM) ANALYSIS TO TA CLONING AND SEQUENCING FOR THE ANALYSIS OF A CLINICAL TRIAL USING AN INVESTIGATIONAL AMINOQUINOLINE, AQ-13, TO CIRCUMVENT CHLOROQUINE RESISTANCE IN SUBJECTS WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN MALI

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Chloroquine resistance, which was first described in Southeast Asia and South America, has now complicated malaria control for more than 50 years. To address this problem, we have developed an analogue of chloroquine (CQ) which is active *in vitro* against CQ-resistant parasites and is safe in human subjects (AQ-13). To test the efficacy and therefore the potential clinical value of this investigational antimalarial, it has been compared for efficacy with the current first-line treatment for patients with uncomplicated *Plasmodium falciparum* malaria (Coartem = Artémether + Luméfantrine, A+L) in a randomized, blinded clinical trial. As part of that process, the efficacy of A+L and AQ-13 has been examined in subjects infected with CQ-resistant vs. CQ-susceptible parasites, based on the K76T single nucleotide polymorphism responsible for CQ resistance. The HRM analyses performed in Mali have shown that parasite isolates obtained from subjects in both groups had specimens with only K76 parasites, only T76 parasites and mixtures of K76 and T76 parasites. Because all subjects in the A+L and AQ-13 treatment groups cleared all asexual parasites from the blood within 3 days, both A+L and AQ-13 were efficacious against CQ-resistant and CQ-susceptible parasites. Based on that information, these samples are now being cloned and sequenced in order to compare the codon present at position 76 of the *Plasmodium falciparum chloroquine resistance transporter* gene (*pfcrt*) in these samples to the results of HRM analyses for the same samples. We anticipate that these results will be available within 2 months and that they will add to the information now available on comparisons of HRM with sequencing for important loci such as position 76 of *pfcrt*.

1501

ARTEMISININ-COMBINATION THERAPY VERSUS CHLOROQUINE FOR THE TREATMENT OF *PLASMODIUM MALARIAE* IN SABAH, MALAYSIA: A RANDOMIZED CONTROLLED TRIAL

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Background. Human infection with *Plasmodium malariae* is uncommon but remains present in the Asia-Pacific region, Africa and South America, and can cause severe anaemia. There have been no previous randomised trials to evaluate the optimal treatment for uncomplicated malaria due to *P. malariae*. Methods. An open-label, randomised controlled trial was conducted at three district hospitals in Sabah, Malaysia. Patients aged 1 year or older with uncomplicated *P. malariae* on screening microscopy were randomly assigned to receive oral artesunate–mefloquine (ASMQ; target dose 12 mg/kg artesunate and 25 mg/kg mefloquine) or chloroquine (CQ; target dose 25 mg/kg). The primary endpoint was parasite clearance at 24 h. Analysis was by modified intention to treat. Secondary analysis

incorporated additional patients separately randomised to artemether-lumefantrine (AL) or CQ. Findings. Between Jan 14, 2013, and Sep 20, 2014, admitted patients with PCR-confirmed *P. malariae* infection were allocated treatment with either ASMQ (n=6) or CQ (n=4). 24 h after treatment, we recorded parasite clearance in 3 (50% [95% CI 12-88]) of 6 patients in the ASMQ group versus none (0% [45–64]) of 4 patients in the CQ group (p=0.091). At 48 hours all ASMQ treated patients were negative for parasites versus none in the CQ arm (p=0.002), with this difference remaining at 72 hours with only 1 patient (25% [0-81]) in the CQ arm demonstrating parasite clearance (p=0.011). Fever clearance appeared faster in the ASMQ arm (median 6 hours [0-18]) versus 18.8 (3.8-42) following CQ (p=0.319). 1 patient in the ASMQ arm developed anaemia at day 28 during follow-up compared to none in the CQ arm (p=0.389). All patients had an adequate clinical and parasitological response to treatment at day 28 of follow-up. There were no serious adverse events due to either study medication. Results were consistent with the larger secondary analysis of patients treated with either ASMQ or AL (n=10) vs CQ (n=10). Interpretation. Artesunate–mefloquine demonstrated a rapid therapeutic response for *P. malariae* malaria, supporting a unified ACT treatment policy for for all *Plasmodium* species in co-endemic areas.

1502

THE BLOOD SCHIZONTICIDAL ACTIVITY OF TAFENOQUINE IS IMPORTANT FOR ITS PROPHYLACTIC EFFICACY

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Tafenoquine is being developed by the US Army and 60 Degrees Pharmaceuticals for malaria prophylaxis. Recent retrospective analyses of clinical data from the Phase II/III program suggest that tafenoquine at the intended dose exhibited similar efficacy to mefloquine, with 100% PE in non-immune subjects and 93% PE in semi-immune subjects, as reported previously. It has been generally assumed that the prophylactic efficacy of tafenoquine was due to the known causal (elimination of developing Pv or Pf hepatic schizonts) or antihypnozoite (killing of latent Pv liver forms) modes of action of the drug. This has led to the suggestion that because of the known association of primaquine relapses with cP450 2d6 polymorphisms, individuals with genetic polymorphisms may also be at greater risk from contracting symptomatic malaria while taking tafenoquine. However, non-clinical studies demonstrating blood schizonticidal effects the similarity of field of efficacy of tafenoquine to mefloquine (per above), the apparent lack of association of *P. vivax* relapses with cP450 2D6 polymorphisms (as reported previously), and findings from clinical and non-clinical studies suggesting merozoite escape from the liver (as reported previously) imply an alternate hypothesis: Tafenoquine also exhibits blood schizonticidal effects that may be important for its prophylactic efficacy in humans.

1503

EVALUATION OF *PLASMODIUM FALCIPARUM* ARTEMISININ RESISTANCE IN WESTERN THAILAND AS PART OF A DOD MULTI-CENTER TRIAL II

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Artemisinin-resistant *Plasmodium falciparum* threatens the effectiveness of all artemisinin-based combination therapies. A multi-center artesunate-mefloquine (A+M) efficacy trial is on-going in three US DoD laboratories in Peru, Kenya, and Thailand, to compare parasite clearance rates at 72 hours after artesunate initiation. Here we report the results of the Thailand site in Sangkhlaburi district near the Thai-Myanmar border. Participants received 4 mg/kg artesunate at 0, 24, and 48 h, 15 mg/kg mefloquine at 72 h, and, at 84-96 h, 10 mg/kg mefloquine plus 0.5 mg/kg primaquine for transmission blocking. To investigate presence of artemisinin resistance (ART-R) by WHO criteria, we calculated parasite clearance half-life (PC1/2), by microscopy per-formed every 4h for the first 12h after first artesunate dose and every 6h for 72h or until two consecutive negative smears. Mutations in the Kelch propeller gene (K13) and 42 day efficacy outcomes were also assessed. Between Oct 31, 2013, and Oct 7, 2015, we enrolled 48 subjects 46 of which were evaluable for at 72h. Of these, 33 (72 %) had K13 wild type (WT) genotypes while 13 subjects (28%) harbored K13 mutations at enrollment, including the commonly de-tected C580Y mutation. The median PC1/2 of the mutant K13 group was significantly longer than the K13 WT group (4.91h 95% CI 4.4-6.5 vs. 3.3h, 95% CI 2.9-4.2, p=0.0003), thus meeting the WHO definition of confirmed ART-R. We also found polymorphisms the MAL genes associ-ated with delayed parasite clearance in nine subjects, including a single subject with both K13 and MAL mutations. Despite this, 100% of subjects achieved adequate clinical and parasitologi-cal response (ACPR) at 42 days. While our data suggest that clinical effectiveness of the tradi-tionally employed 3 day A+M regimen has yet to be compromised, ART-R is now confirmed in this area of western Thailand.

1504

PREVENTION OF MALARIA IN PREGNANCY: QUANTIFICATION OF TARGET CONCENTRATIONS OF DIHYDROARTEMISININ - PIPERAQUINE

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In a recent randomized trial comparing intermittent preventive therapy (IPTp) regimens to prevent malaria in 300 pregnant women in Tororo, Uganda, dihydroartemisinin-piperaquine (DP) given once a month (DPqm) or every 2 months (DPq2m) was superior to sulfadoxine-pyrimethamine (SP) given every 2 months (SPq2m), and DPqm was superior to DPq2m for several outcomes. The aim of this analysis was to quantify preventive (target) concentrations of piperaquine (PQ) using population pharmacokinetic (PK) –pharmacodynamic (PD) analysis, with the presence of circulating malaria parasites during pregnancy (PD endpoint) detected by a highly sensitive loop-mediated isothermal amplification (LAMP) assay. All women contributed longitudinal PD data (2260 observations obtained at monthly routine visits and at the time of clinical malaria), and women receiving DP contributed longitudinal PK data (1293 venous and capillary measurements obtained at monthly routine visits). Nonlinear mixed effect modeling was used for joint continuous (population PK) data and repeated binary measurements (LAMP) analysis. The probability of parasitemia was 46% (relative standard error RSE, 39%), 22% (RSE 9%) and 11% (RSE 5%) in the SPq2m, DPq2m and DPqm arms, respectively. More frequent DP dose (DPqm) was associated with absence of malaria parasites ($p<10^{-21}$), however PQ plasma levels were far superior to DP dose interval ($p<10^{-95}$) as a predictor of parasitemia; PQ plasma concentrations of 5.5 ng/mL, 8.7 ng/mL and 13.3 ng/mL were found to provide 95%, 99% and 99.9% protection from parasitemia. Modeling target concentrations, 90% of women in the DPq2m arm, but only 15% of those in the DPqm arm had PQ levels below 5.5 ng/mL for at least 25% of time receiving IPTp. Population clearance of PQ in Ugandan women was 3140 L/day (RSE 6%), with modest between subject variability (CV % 35 (RSE 13%). There were no changes in PQ PK during the course of pregnancy. In conclusion, our

analysis provides evidence to define the plasma level of PQ that prevents malaria parasitemia during pregnancy, and offers a rational framework for further optimization of dosing strategies of DP in IPTp regimens.

1505

USING POLYPHARMACOLOGY TO IDENTIFY NOVEL DRUGS AND DRUG TARGETS AGAINST MALARIA INFECTION

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Malaria control and eradication efforts are hindered by drug resistance, difficulty targeting liver-resident hypnozoites and a prolonged process of de novo drug development. Chemotherapeutics which target host proteins within liver-stage infected hepatocytes have the capacity to overcome each of these roadblocks. We have recently demonstrated that targeting the hepatocyte P53 and Bcl-2 pathways can eliminate multiple species of Plasmodium parasites, including *P. falciparum*, from the liver. To expand the range of host-targeted drugs, we have applied a systems biology approach that identifies critical host kinases for the development of Plasmodium liver stage. Our approach takes advantage of 37 broad-spectrum kinase inhibitors and their measured activity against 301 human kinases. By assessing the efficacy of these 37 inhibitors against liver stage infection, and training a model based on machine learning algorithms, we predicted 33 kinases with the largest role in liver stage infection. These kinases include several Receptor Tyrosine Kinases, and members of the Protein Kinase C cascade. This also includes five out of eight of the kinases identified by Prudêncio and colleagues in their siRNA screen (P=0.0005) for host kinases involved in liver stage infection. Moreover, this platform allows for the prediction of novel kinases inhibitors, including those already tested in the clinic for other indications. If effective, these compounds might provide a rapid path to new anti-malarials for prophylaxis and radical cure. Since this approach identifies key signaling networks from a condensed data set, it's compatible with freshly isolated parasites from the field, as well as *in vivo* screening approaches. Our ongoing efforts include applying this approach to rapidly identify novel inhibitors against a variety of intracellular parasites and bacteria and also to emerging infections such as Dengue and Zika viruses.

1506

DOXYCYCLINE TARGETS THE BACTERIA-LIKE SMALL SUBUNIT RIBOSOMAL RNA IN THE *PLASMODIUM FALCIPARUM* MALARIA PARASITE

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Doxycycline is a tetracycline-class antibiotic that is highly effective for malaria chemoprophylaxis and is also used in combination with quinine for malaria treatment. In bacteria, like other tetracyclines, targets of doxycycline include the small subunit ribosomal RNA (SSrRNA) molecule and proteins within the 30S small subunit ribosome, which are involved in protein synthesis. However, the mechanism of action of doxycycline against the malaria parasite is uncertain. Evidence suggests that doxycycline inhibits protein synthesis within the plasmodial apicoplast, whose extranuclear genome contains homologues to bacterial genes. As a means to identify a molecular target(s) and the mechanism of action in *Plasmodium falciparum*, we attempted to select for doxycycline resistance. After several months of *in vitro* culture under continuous incremental doxycycline pressure, we generated resistant parasites. Clonal parasite lines showed stable and significant increases in the IC_{50} to doxycycline, determined by a 96-hour growth inhibition assay necessary to capture the slow acting schizonticidal activity of the cyclines. Sanger sequencing of the apicoplast small subunit ribosomal RNA gene (*pfssrRNA*; PFC10_API0057) revealed novel SNPs in resistant parasites. In the absence of drug pressure, doxycycline resistant parasites exhibited slower growth compared to controls, suggesting a fitness cost accompanies resistance. Our results suggest that in malaria parasites, doxycycline targets the plastid-encoded, bacterial-like SSrRNA. Similar to the action of cyclines against bacteria, doxycycline may prevent binding of an incoming aminoacyl-transfer RNA to the A site of the ribosome and thus block the elongation step of protein synthesis in the parasite apicoplast.

1507

ENHANCING TRANSLATIONAL SIGNIFICANCE OF *PLASMODIUM FALCIPARUM* MOUSE MODEL

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Malaria continues being a major global disease and current therapies are threatened by spread of resistant parasites. This situation has prompted antimalarial community to identify new molecules suitable to be used as part of new treatments that can overcome current issues. The last few years have seen unprecedented progress in the identification and early clinical testing of novel antimalarial drug candidates. However effective progression of candidate antimalarials depends on selecting optimal dosing for clinical studies. Understanding and determining efficacy parameters using preclinical models is critical to estimate effective human doses. One of the most important parameters is to estimate the parasite reduction rate (PRR) to determine how long efficacious drug concentrations should be present to fully eliminate blood parasites and cure the patients. Significant advances have been done in this field during the last years with the development of *in vitro* assays that can determine the killing profile of antimalarial compounds (1). However, a quantitative *in vivo* assay to determine rate of parasite killing was missing. We have adapted the *Plasmodium falciparum* mouse model protocol to allow determining simultaneously rate of clearance and killing *in vivo* by antimalarial drugs. These studies provide invaluable results that can be used for a robust estimation of PRR in patients to inform human dose predictions. Such data can inform clinical trials required for effective deployment of novel antimalarial treatments.

1508

DETECTING ANTIMALARIALS IN BLOOD FROM COMMUNITY SURVEYS IN TANZANIA

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The assessment of the impact of diagnostic or treatment strategies on antimalarial drug use often rely on histories of drug intake in community surveys. Estimating accurately the levels of circulating antimalarials in a population allow an unbiased measure of drug consumption. Here, we explored the relationship between endemicity, health facility practices and blood drug concentration in malaria-endemic communities. The study took place in three regions of Tanzania (Mwanza, Mbeya and Mtwara) in

2015. In each region, surveys were conducted in three districts. All health facilities from randomly selected wards were visited to assess treatment and diagnosis practices. Information on demographics, health seeking behavior and drug use was obtained through community surveys, while health facility based surveys collected information on diagnosis and treatment practices. Finger-prick blood samples were obtained for on-site testing as well as for collecting samples on filter paper. Antimalarial blood concentration including Lumefantrine was measured later by LC-MS/MS. Parasite prevalence was 20% (506/2463) in Mwanza, 4% (84/1985) in Mbeya and 26% (559/2152) in Mtwara. Participants with any antimalarial in the blood were 34% (844/2463), 20% (399/1985) and 26% (554/2152) respectively in Mwanza, Mbeya and Mtwara. Individual who tested positive with a RDT had a marginally higher frequency of being detected with an antimalarial (29.7% vs 26.8%). Indeed, the association of RDT positivity and presence of drug was relatively poor (OR=1.15, 95% CI:1.00-1.33). Results from health facilities showed that amongst the febrile patients, 67% (151/226) were tested for parasite. Prevalence of persons with residual antimalarials in the blood was relatively high, especially in Mbeya where endemicity was low. This indicates poor use of diagnostic testing and inappropriate prescription of antimalarials. This might result in high levels of circulating drug in a population, probably the most important driver of the development of drug resistant pathogens. Effort should therefore be made to reduce these poor practices and prevent emergence of resistance.

1509

ACTIVE DETECTION OF ASYMPTOMATIC MALARIA BY LOOP MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) IN NORTHWEST ETHIOPIA

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Despite the scale-up of universal intervention measures, asymptomatic malaria is one of the challenges that needs to be addressed in malaria endemic sub-Saharan Africa. Due to the poor sensitivity of common laboratory diagnosis methods of malaria like rapid diagnosis tests (RDTs) and microscopy, field deployable rapid molecular techniques need to be available to accurately diagnose malaria. This pilot study aimed to assess the performance of loop mediated isothermal polymerase amplification (LAMP) for the detection of asymptomatic malaria in North Gondar, Ethiopia. A community based cross-sectional study was conducted from February to May 2014 in North Gondar, Ethiopia. A total of 802 study participants were enrolled. Data on socio-demographic profile and associated risk factors for asymptomatic malaria were collected using interview-based questionnaire. Capillary blood was collected and blood films and dried blood spots (DBS) were prepared for malaria parasite detection with giemsa microscopy, nested polymerase chain reaction (nPCR) and LAMP using a non-instrumented nucleid acid amplification (NINA) device. In this study, 45.3% of the study participants had access to combined universal intervention measures of malaria. LAMP and nPCR were performed on 160 DBS samples. The overall prevalence of asymptomatic malaria using giemsa microscopy, LAMP and nPCR was 3.75%, 5% and 4.375%, respectively. In conclusion, LAMP is able to identify two extra asymptomatic malaria carriers per 100 study population. This study indicated that active diagnosis of asymptomatic malaria with low-cost techniques like LAMP can support malaria elimination through enhanced active case detection.

1510

EVALUATING THE COSTS AND COST-EFFECTIVENESS OF MICROSCOPY COMPARED TO LAMP USED DURING REACTIVE CASE DETECTION IN ACEH PROVINCE, INDONESIA

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Reactive case detection (RACD) is an active surveillance method where households and neighbors of a passively identified case (an index case) are screened to identify and treat asymptomatic infections with the goal of reducing further transmission. Despite being a resource-intensive activity, RACD is recommended and implemented widely in malaria-eliminating countries. However, little is known about the cost to conduct RACD and the cost effectiveness of the various diagnostic methods used during RACD to identify otherwise undetected cases. The aim of this study was to determine the cost of conducting RACD, and compare the cost effectiveness of microscopy (currently standard practice) to the more sensitive loop mediated isothermal amplification (LAMP) as a diagnostic method. The study site included five subdistricts in Aceh Besar District, Aceh, an eliminating province located on the westernmost tip of Indonesia. Costs and effects were recorded for a 20 month study period between May 2014 and December 2015. The cost of all inputs used in conducting RACD (capital, consumables, personnel, services) was recorded. A total of 38 passively-detected malaria cases were recorded during the study period. Using standard procedures, these index cases were traced back to their community and an additional 1,495 individuals were screened. The average cost of conducting RACD using microscopy and LAMP was $2,481 per event with personnel being the main cost driver. The average cost of screening each individual during RACD was $46.96, with diagnostic testing costs $0.54 and $12.26 per person for microscopy and LAMP, respectively. Among the actively screened people, two new cases were detected by microscopy, confirmed by LAMP, and an additional four cases were detected only by LAMP. The incremental cost effectiveness ratio of LAMP versus microscopy was estimated to be $4,383 per case detected. LAMP was more costly, but more sensitive for the detection of additional cases in RACD and may play an important role in detecting and treating the asymptomatic reservoir and reducing onward transmission in eliminating settings.

1511

A FIELD-BASED POINT-OF-CARE ASSAY TO DETECT ANTIMALARIAL DRUGS FROM FINGERSTICK BLOOD SAMPLES

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Malaria parasites with reduced sensitivity to several of the current first-line antimalarial drug therapies - artemisinin-based combination therapies (ACTs) - have recently emerged in Cambodia. Preventing the spread of drug resistant parasites through Southeast Asia and to Africa is a top priority for global malaria elimination campaigns. The ability to detect these small molecule drugs in malaria patient samples at the point-of-care would allow healthcare workers to identify previous treatment failures and adjust future treatment to improve efficacy and reduce the spread of resistant parasites. A simple, point-of-care assay to detect ACT partner

drugs would also allow for real-time mapping of drug use and distribution. We aim to develop a low-cost, field-based test to detect several slow-clearing ACT drug compounds from fingerstick blood samples. We will select drug specific aptamers via an inverted SELEX protocol in which we immobilize a DNA library and isolate structure-switching sequences that are released upon binding drug target in solution. Our assay will filter out blood cells and provide a colorimetric readout of drug levels in recovered plasma via the interaction of drug, aptamer, and colloidal gold. In order to remove subjectivity associated with user interpretation of the assay, we will develop a smartphone application to quantify colorimetric readout, storing results along with location and patient information. We will design this device with constant input from clinicians and healthcare workers to ensure its feasibility for use in rural clinics in malaria-endemic settings.

1512

FIRST NATIONAL INTEGRATED COMMUNITY CASE MANAGEMENT (ICCCM) ONSITE TRAINING AND SUPPORTIVE SUPERVISION ASSESSMENT IN GHANA IN JANUARY-FEBRUARY 2015

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Integrated Community Case Management is an intervention to get sick children under five years of age treated at the community level as quickly as possible for malaria, cough or difficulty in breathing and diarrhoea. An Integrated Community Case Management (ICCM) Onsite Training and Supportive Supervision (OTSS) assessment was carried out in the months of January and February 2015.This became necessary because there were a number of challenges relating to community-based agents (CBAs) with some districts not reporting at all or on time. This had led to the inability to meet set targets of the program for the year 2014. Community health nurses and officers (CHNs/CHOs) (direct supervisors of CBAs) were trained and sent on the field. In the field CHNs/CHOs carried out training and supportive supervision using the OTSS checklist. After the assessment, members of the district health management team (DHMTs) with National teams and supervisors discussed findings and planned possible solutions for the challenges encountered. At the national level, the data was entered using epi info software, collated, cleaned and analysed. In all 10,393 CBAs were assessed. CBAs were able to diagnose and treat malaria and diagnose diarrhoea effectively using symptomatic diagnosis. Most CBAs, who had been trained on malaria rapid diagnostic tests (mRDTs), could not carry out the test. CBAs also had difficulty in correctly assessing for cough and difficulty in breathing in terms of counting breaths. There was erratic availability of artesunate-amodiaquine for malaria treatment. There were little or no medicines managing cough and difficulty in breathing and diarrhoea; even in regions that were expected to have supplies. Referrals by CBAs were done but caregivers generally refused to send their children to the next level of care. Most CBAs require training, as a lot of them had their training. In conclusion, CBAs are able to diagnose and treat malaria and diagnose diarrhoea symptomatically. They however lack the capacity to use mRDTs. They also need more training on diagnosis of cough and difficulty in breathing. The CBAs also need to be updated.

1513

THE ROLE OF MOBILE DEVICES IN RAPID DIAGNOSTIC TESTING QUALITY CONTROL ON COMMUNITY HEALTH VOLUNTEERS IN WESTERN KENYA

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Community health volunteers (CHVs) are trained to provide basic disease prevention and health promotion at the household level. In order to improve early diagnosis of malaria amongst rural populations, CHVs are an avenue to provide basic malaria testing with rapid diagnostic tests (RDTs) and treatment for uncomplicated cases. Monitoring RDT performance of CHVs is vital in ensuring quality and accuracy of testing. Physical monitoring of CHVs in real time is complicated in large-scale programs, thus the need for innovative quality control approaches. As part of a larger study, we trained a total of 275 CHVs on how to perform malaria RDTs. We introduced Fio Corporation's android based mobile diagnostic devices (Deki™ Readers) to remotely monitor the performance of a sample of CHVs in real time. These Deki Readers provided technological support for quality control by identifying RDT processing errors (too much/too little buffer, sample or buffer in wrong well, too much/too little blood) and interpreting rapid diagnostic tests (positive/negative/invalid). For our study Deki Reader software was programmed to interpret the tests only after the CHV provided their interpretation allowing for comparison between Deki Reader and CHV interpretation and real-time feedback to the CHV. Both results were uploaded to a secure data server for real-time review by the study team. The CHVs requiring additional mentorship were identified and followed up. Ninety (90) CHVs were randomly selected and trained for two days. CHVs also received technical support from Fio support staff. A total of 1199 clients were tested where 89.66% (1075) of the tests were concordant, 5.17% (62) tests had interpretation errors while 5.17% (62) had processing errors. The mean age of the CHVs was 41.4 years (C.I 40.9 - 42.0). 53.42% (47) of the CHVs had secondary education and above. Error rates were not correlated with age and education level. The low error rates irrespective of age and education is encouraging and confirms the ability of CHVs to correctly perform RDTs. Mobile devices to monitor CHV performance are feasible and valuable in the quality control of malaria diagnosis in remote areas.

1514

DETECTION OF MALARIA INFECTION BY HEMOZOIN CONTENT COMPARED TO RDTS AND MICROSCOPY FROM PERUVIAN AMAZON SAMPLES

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Detection of the presence of malaria parasites primarily relies upon the use of RDT card tests or microscopy-based methods. Microscopy testing time is 30-60 minutes per sample and requires skilled readers. RDT testing time is 15-20 minutes, and sensitivity is lower in some species and variants. There is a need for novel malaria diagnostic techniques to rapidly and accurately diagnose across all species and with HRP2 deletion. A multidisciplinary effort designed an inexpensive, rapid (one minute) malaria detection device that indicates the presence of hemozoin, a by-product of parasite digestion of hemoglobin. Polarized laser light passing through a blood sample is used to diagnose malaria. When the partially magnetic malaria

hemozoin is present in a blood sample, it aligns with a magnetic field decreasing the amount of polarized laser light able to pass through it. This decrease in light is directly proportional to parasitemia (R2=0.996). A test of 69 patients in Peru comparing an early prototype to microscopy achieved 92% sensitivity and 100% specificity for *Plasmodium falciparum* and *P. vivax* infections. Comparing CareStart RDTs to microscopy for these same samples showed 67% sensitivity and 100% specificity. The decreased effectiveness of RDTs in Peru is likely because of known HRP2 deletions in Peruvian *P. falciparum* and higher prevalence of *P. vivax* which is difficult to detect with RDTs. Our long term goal is to translate this technology into a robust, low-cost device, which can be used in malaria-endemic regions to enable rapid malaria diagnosis at the point-of-care for all species of malaria.

1515

PLATFORM FOR *PLASMODIUM* DETECTION IN BLOOD DONORS FROM ENDEMIC AND NON-ENDEMIC BRAZILIAN AREAS: PROCESSING OF POOLED SAMPLES USING MOLECULAR AND SEROLOGICAL MARKERS

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Malaria transmitted by blood transfusion remains one of the most important infections for hemotherapy services. In Brazil the incidence of malaria by blood transfusion is unknown, and this event may contribute to the spread of the disease in cases of failure in the clinical and epidemiological screening or due to asymptomatic donors. Donors that caused transfusion malaria showed very low parasitemia with an estimated rate of 1 to 10 parasites per unit of blood, which requires sensitive methods for the diagnosis and prevention. This study included 147 Brazilian public or private blood banks located in endemic and non-endemic areas for malaria with 13,383 blood donors that were accepted by the local methods of screening. The samples were grouped into pools of 10 and were processed by three different real time PCR and one nested PCR. A rapid test It was used for antibody detection. Samples from the positive pools were tested individually by PCR to detect positive donors. Real-time PCR revealed amplification for Plasmodium in 43 pools with samples from Amazon Region (4.72%) and Extra-Amazon Region (3.19%). Nested PCR detected four pools with *P. vivax*, two pools with *P. falciparum* and one pool with *P. malariae*, all related to samples from Extra-Amazon Region. Samples from positive pools were processed individually and real-time PCR revealed amplification in 25 donors, showing a positivity rate of 6.94% in 360 individual samples. Nested PCR detected two donors, one harboring *P. malariae* and one *P. vivax*. The rapid diagnostic test was positive for *P. vivax* in 13 pools from non-endemic area and in three pools from endemic area. Real-time PCR from Lima showed the best performance and was able to identify Plasmodium in pools of 10 samples, reducing time and cost of processing. This study, which analyzed blood samples from donors from endemic and non-endemic areas revealed the risk of transfusion malaria in our country and the need for sensitive validated protocols for detection of low parasitaemia. These results may support the decision making of blood donor screening criteria by regulatory agencies, in order to reduce malaria transmission in Brazilian blood banks.

1516

REDUCING THE DIAGNOSTIC BURDEN OF MALARIA USING MICROSCOPY IMAGE ANALYSIS AND MACHINE LEARNING IN THE FIELD

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Microscopy remains the main technique for diagnosing malaria, despite the availability of Rapid Diagnostic Tests. Hundreds of millions of blood films are examined using microscopy every year for diagnosing malaria and quantifying parasite burdens. Processing this large number of slides consumes scarce resources. Microscopy technicians who read these slides in the field may be inadequately trained or overwhelmed with the volume of slides to process, leading to missed and incorrect diagnoses. To ease the burden for microscopists and improve diagnostic and quantitative accuracy, we have developed a smartphone application that can assist field microscopists in diagnosis of malaria. The software runs on a standard Android smartphone that is attached to a microscope by a low cost adapter. Images of thin-film microscope slides are acquired through the eyepiece of the microscope using the smartphone's built-in camera. The smartphone application assists microscopist in detecting parasites and estimating the parasitaemia. For each microscope field, the image processing software identifies infected and uninfected cells, and reports the parasite count per microliter of blood. The software was trained with more than 200,000 red blood cells from slides acquired at Chittagong Medical College Hospital in Bangladesh from patients with and without *Plasmodium falciparum* infection. These were manually annotated by an experienced professional slide reader. This is one of the largest labeled malaria slide image collections, enabling the application of new machine learning techniques such as deep learning. For each field-of-view image taken, an image processing pipeline is applied first to detect and segment cells before computing color and texture features for automatic machine classification to discriminate between infected and uninfected cells and other objects in the slide. Initial experiments show that our software correlates highly with both human experts and flow cytometry. We demonstrate the smartphone user interface, show the typical smartphone application work flow, and report on the diagnostic performance in field conditions in Bangladesh.

1517

REAL-TIME QUALITY ASSURANCE OF MALARIA SURVEILLANCE DATA IN MYANMAR AND ITS BORDER WITH CHINA

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Collection of high quality data in public health surveillance and research studies is essential to ensure data interpretability and applicability. We evaluated the feasibility of systematic quality assurance procedures and the impact of a real-time integrated approach for large-scale malaria surveillance in Myanmar. We will present the data from and experience with evidence-based integrated monitoring and evaluation (M&E) procedures, with real-time feedback and quality assurance, implemented during the preparation and conduct of a large malaria surveillance study. The study was conducted in 43 villages located in 13 rural malaria-

endemic townships of nine State and Regions of Myanmar, and by a network of seven research and non-research partners working in the public and private sectors on malaria control and/or surveillance towards malaria elimination. M&E procedures were developed and performed manually and electronically using an edit-check system. Training was provided before and again during the study, with quality assessments before and after mid-study retraining. Study documents were comprehensively and systematically reviewed. Sample quality was evaluated by a trained team of laboratory experts, using a pre-specified check list. The most common and critical errors were site- and partner-specific, regardless of the type of the study. Documentation errors were related to age, travel history, antimalarial treatment history, evidence of consent, sample labeling, the quantity of blood required, and sample contamination. Findings from a comprehensive evaluation of approximately 5,000 study documents and more than 12,000 blood samples, using three different types of study record forms, and the quality before and after re-training, will be presented. Systematic and comprehensive monitoring and evaluation of data and related samples can be effectively integrated within a surveillance system, and real-time evaluation and feedback early in the process may significantly improve the quality of the data and samples, therefore subsequent usefulness of public health interventions developed and deployed based on these data.

1518

PLASMA-QPCR FOR DIFFERENTIAL DIAGNOSIS OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX*

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Accurate, sensitive detection of human malaria parasite, by species, is required for the treatment of the disease, and for assessing the parasite load in asymptomatic carriers. Microscopy and antigen-based rapid tests reliably detect upto 50,000 parasites per ml of blood. Newer nucleic acid amplification methods offer higher sensitivity and flexibility but use template preparations that are laborious, expensive and subject to carryover sample contaminations. We use as little as 4 µl of patient plasma as a source of parasite DNA, without a need for purification. This molecular diagnosis method is simple, rapid, sensitive and reliable. Brand new sets of primers for the detection of *Plasmodium falciparum* (Pf) and *P. vivax* (Pv) by qPCR were designed at a non-conserved region of 18S ribosomal RNA genes. Species specificity of designed primers were confirmed by bioinformatics, by biochemical validation and by Sanger sequencing of amplified products. Though the presence of plasma delayed Ct value by four cycles with each tested standard genome sample, the linear range of detection was identical to reactions without plasma. With more than 90% amplification efficiency, the limit of detection stood less than 50 copies of parasite genome per ml of blood. Over 200 diverse patient samples were tested in triplicates on a 96-well qPCR instrument in parallel using Pf or Pv primer sets, with positive and no-template control reactions. Around 84 % of the plasma-qPCR results agreed with both microscopy and RDT, 9 % agreed with one of the two methods, 6 % displayed higher sensitivity and 1 % of the samples did not agree any of the two methods. This is better than any published comparison of three different methods. Direct plasma-based molecular diagnosis opens new avenues for differential screening of suspected malaria samples.

1519

QUALITY OF FEVER CASE MANAGEMENT IN THE PRIVATE SECTOR IN KINSHASA: RESULTS FROM BASELINE EXIT INTERVIEW AND MYSTERY CLIENT STUDIES

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The private sector is the most common fever treatment source in Kinshasa. 70% of childhood fevers are treated in this sector and 97% of antimalarials are distributed through private outlets, including 89% through unregulated drug shops where diagnostic testing is not available. There is no published data on the quality of fever case management in private outlets, and a pressing need to fill this gap. As part of a project to increase RDT availability and correct use in this sector we conducted exit interview and mystery client surveys to benchmark standard quality of care indicators in late 2015. 123 mystery client visits by confirmed RDT-negative volunteers were conducted at 65 facilities with blood testing available. 1,655 eligible client interviews were conducted at 83 health facilities, 44 pharmacies and 60 drug stores. Eligible clients were adults seeking treatment for fever for themselves or on behalf of someone else. 79.3% of facility clients received a malaria test, with 26.7% tested by RDT and 11.4% tested by both RDT and microscopy. 82.8% of test-positive facility clients received any antimalarial. However, fewer than half received an ACT (43.9%) and a similar proportion received both an antimalarial and an antibiotic (43.7%). The most common non-ACT treatments were quinine and artemisinin-based injections. 19/46 (41.3%) test-negative clients received any antimalarial. Testing was uncommon in pharmacies and drug shops (<8%) and 4 out of 10 untested clients received any antimalarial (40.6%). Mystery clients experienced poor quality of case management for fever at facilities. In 63% of visits the provider reported the client was positive for malaria following testing, and only 10% of clients received the correct diagnosis (negative) and did not receive any antimalarial. Providers wore gloves for only 23% of tests and waste disposal was suboptimal (immediate disposal of lancet in sharps box: 65% of tests). These results confirm there is much scope for improving private sector fever case management in Kinshasa, including both the provision of testing and availability of quality-assured ACT treatment.

1520

ANTIBODIES TO *PLASMODIUM VIVAX* MSP1-19 RECOMBINANT ANTIGEN IN BLOOD DONORS FROM SAO PAULO BLOOD BANK

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Transfusion-transmitted malaria (TTM) is a challenge for blood banks due to asymptomatic *Plasmodium* infections. In non-endemic and low endemic areas, TTM is a rarely reported event. Sao Paulo state is not endemic for malaria, but sporadic autochthonous cases, mostly asymptomatic, have been reported and transfusional cases due to asymptomatic donors harboring *P. malariae* have been described. These donors involved in TTM were individuals who had visited the Atlantic Forest in different regions of the state and were unaware of their *Plasmodium* carrier status. The aim of this study was to evaluate the exposition of donor candidates to *Plasmodium* as a measure of TTM risk. Blood samples were collected from 6,328 candidates for blood donation that attended Fundacao Pro-Sangue/Hemocentro de Sao Paulo. IgG antibodies were surveyed in plasma samples by ELISA using *P. vivax* $MSP1_{19}$ recombinant antigen. The reactivity index (RI) was calculated for each sample and values of RI ≥ 1.0 were considered positive. Out of 6,381 donors, 51 were positive

for IgG anti-PvMSP1$_{19}$ (0.81% - IC 95% = 0.61-1.06). RI≥1.0 varied from 1.01 to 9.92 (media=3.15 and median=2.26). Among the 51 samples positive by serology, 68.63% were from donors with displacements to the Atlantic Forest biome in São Paulo State and/or who live in regions near the Atlantic Forest. Using molecular tools, researchers have detected that approximately 50% of *Plasmodium* infections related to Atlantic Forest biome are caused by *P. vivax* and 50%, to *P. malariae*, corroborating our results. The detection of anti-*P. vivax* IgG in blood bank donors in non-endemic area is a measure of the exposure of candidates to *Plasmodium* antigens, but not necessarily a marker of parasitemia or disease. These results constitute an alert as asymptomatic donors are currently missed by the clinical-epidemiological screening preceding donation and no laboratorial test is applied. This study is part of a project that evaluates the power of a few specific questions on donor's proximity to the Atlantic Forest in addition to laboratorial methods to disclose *Plasmodium* carriers, aiming to reduce the risk of TTM in this area.

1521

ASSESSMENT OF THE VARIABILITY IN THE INTERPRETATIONS OF MALARIA RAPID DIAGNOSTIC TEST (MRDT) RESULTS

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Malaria RDTs are accurate and relatively simple to use. However, correct processing and interpretations of the test results are critical for mRDTs to perform optimally in the field. Errors in the post-processing phase, properly interpreting the RDTs as positive, negative or invalid, remain frequent. Based on 4 field studies of FIonet, where a healthcare worker and an automated RDT reader provided mRDT result interpretations, discordance was estimated to occur in ~9% of mRDTs. Our aim was to investigate the discordant results, to assess the variation and repeatability of the interpretation of the mRDT results. We developed a questionnaire composed of high-resolution images of mRDTs conducted in the field by healthcare workers during the Fionet studies, where an image of each RDT processed was captured at the time for interpretation. A proportional stratified random sample of images was selected, where the healthcare worker and Fionet had discordant interpretations of the mRDT results. Additionally, 2 mRDTs were included as controls and 2 were repeated. Respondent characteristics, such as their level of experience were collected. So far 45 respondents have completed the survey, with ~26% being experienced RDT users and located across 3 continents. Repeated mRDT images demonstrated that over 10% of respondents changed their interpretation between the first and second appearance of the image in the survey (Kappa=0.73). A large amount of variability in the interpretations was observed, from complete agreement to almost complete disagreement, for example the respondents' results for one mRDT where 44% positive, 46% negative and 10% invalid. For >30% of the mRDT images, there was less than 75% agreement on the interpretation. The largest variation in interpretation was observed when there was a very faint line present. Interpretations were not statistically different between experienced and non-experienced RDT users. The reliability of the interpretations of the ~9% of mRDT test results, are variable and consequently sub-optimal to rely on user interpretations in the field and supports the implementation of an objective automated RDT reader.

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PUNCH CARD MICROFLUIDICS PLATFORM FOR MULTIPLEX MOLECULAR DIAGNOSIS OF *PLASMODIUM FALCIPARUM* AND *P. VIVAX*

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Malaria continues to cause massive devastation globally. Routine microscopy and RDTs often fall short in diagnosing cases with low parasite densities. Moreover, with current elimination efforts in countries that are prime for such, increasing global concerns surrounding antimicrobial resistance and presence of co-infections, there is a need for effective multiplex diagnostic tests that can detect low parasitemia levels. To address this need, we have invented a punch card-based microfluidic platform and are using it to implement multiplex molecular diagnostic assays for malaria. Specifically, we are implementing recombinase polymerase amplification (RPA) assays starting with *Plasmodium vivax* and *P. falciparum*. Our platform is a self-contained, completely integrated hand-crank powered, programmable microfluidic platform. A paper tape encodes information as a series of punched holes. A mechanical reader/actuator reads these paper tapes and correspondingly executes operations onto a microfluidic chip coupled to the platform in a plug-and-play fashion. Enabled by the complexity of codes that can be represented by a series of holes in punched paper tapes, we harness our 30 independently controlled pumps and valves to implement the assays. Unlike conventional lateral flow-based tests, our platform has the capacity to process larger sample volumes, run multiple steps with the capacity to incorporate wash steps and the capability of implementing quantitative results if needed. Nucleic acid extraction is achieved using Fusion 5, a glass microfiber membrane that is embedded in the microfluidic chip. To achieve the optimum temperature of 37 degrees Celsius for the RPA assays, a heating pad is implemented in the device using a method that is similar to the previously described non-instrumented nucleic acid amplification (NINA) approach. With its portable and robust design, low cost and ease-of-use, we envision punch card programmable microfluidics bringing complex control of microfluidic chips into field-based diagnostic applications in low-resource settings to help combat malaria.

1523

SUPPORTING THE IMPLEMENTATION OF MALARIA RAPID DIAGNOSTIC TESTS (RDTS): TOOLS FOR QUALITY CONTROL AND ASSESSMENT IN ENDEMIC SETTINGS

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The World Health Organization (WHO) recommends that every suspected malaria case be confirmed by parasitological testing using microscopy or malaria Rapid Diagnostic Tests (RDTs), and treatment of confirmed cases be done with artemisinin combination therapies (ACTs). Therefore there is a need for good quality RDTs to ensure access to prompt and accurate diagnosis, especially in remote settings where good quality microscopy may not be available. The need for RDT quality and durability, especially under transport and storage conditions typical in malaria-endemic regions, has received considerable attention. Early evidence has shown lot to lot variation of products and susceptibility to deterioration upon exposure to high temperatures and humidity which can be encountered along the supply chain. Moreover documented reports show that health worker's poor adherence to RDTs test results is partly attributed to lack of confidence in the quality of tests. Tools to monitor the quality of RDTs at the end-users level and manage any problems have been set up in the frame of an RDT implementation project, with inputs from various implementers as well as health care staff from public and private sectors. A protocol was developed for countries to have a framework in which RDT problems will be documented, cross-checked and reported from the end user to the national, regional and international stakeholders. A troubleshooting guide provides help to RDT users and their supervisors to address problems that occur during use of RDTs. Positive control wells (PCWs) have been made available for point-of-care (POC) users, to reassure them on the quality of the test kits. When reconstituted with water and applied to a good quality RDT, the PCW solution produces a

positive test result. PCWs can therefore be used as POC quality control tool by front-line health workers to test their RDT stocks and ensure their validity and accuracy. Preliminary results from a pilot study assessing the use and acceptability of PCWs, the troubleshooting guide, and the problems protocol by health worker supervisors in the private and public health sectors in Kenya and Tanzania will be presented.

1524

PERCEIVED VALUE OF MALARIA RAPID DIAGNOSTIC TESTS AMONG PRIVATE PROVIDERS IN MADAGASCAR AND UGANDA: A QUALITATIVE STUDY

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Malaria rapid diagnostic tests (RDTs) have become ubiquitous in public sector health facilities throughout the malaria-endemic world, but private sector use is still nascent. Little is known about private provider perceptions of RDT credibility, how RDT results affect treatment decisions, and whether providers see RDTs as an asset or liability from both a health and a business perspective. We conducted in-depth interviews (IDIs) with 36 private providers in Uganda and 36 in Madagascar, most enrolled in a private-sector mRDT promotion program. IDI topics included RDT use, perceived test credibility, alternative diagnostic strategies, treatment decisions, pricing strategies, and perceived effect on overall profitability. Most providers agreed that RDTs improved their profitability and standing in the community. However, both confidence in test results and treatment based on test results varied widely. This presentation will describe the range of private-sector provider perspectives about RDT credibility and situations in which providers do or do not base treatment on RDT results. It will also consider the implications of study findings for scaling up RDT use in the private sector. This qualitative study was part of a three-year initiative to pilot RDT use among private providers in Kenya, Madagascar, Nigeria, Uganda and Tanzania.

1525

FIVE YEARS INTO THE MALARIA DIAGNOSIS SCALE-UP, ARE ACTS REALLY GETTING TO INFECTED PEOPLE? ESTIMATING ACT MISUSE IN THE INFORMAL PRIVATE SECTOR

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In 2010, the World Health Organization broadened its recommendation to parasitologically confirm malaria before treatment is provided, catalyzing the increased availability and use of malaria rapid diagnostic tests (mRDTs). The use of mRDTs promotes appropriate treatment for febrile illness and prevents antimalarials, especially the first line artemisinin-based combination therapies (ACTs), from being wasted on patients without malaria. Even so, mRDTs are not always available in the private health sector, where a substantial proportion of fever patients seek treatment, contributing to the irrational use (or misuse) of antimalarials, especially in the informal private sector, i.e. drug shops. A model to quantify ACT misuse will help in setting priorities as further scaling-up of mRDTs occurs in endemic contexts. The following data inputs were used in the model to estimate ACT misuse in a given geography: malaria prevalence, number of fever patients seeking treatment, proportion of fevers receiving a malaria test, proportion of fevers with or without a test, and proportion of fevers receiving ACTs. These inputs were derived from raw data or modeled from the Demographic Health Surveys, ACTWatch Surveys, reviews of published literature, and data from the national malaria control programs. The number of ACTs misused was estimated as the number of ACTs given to fever cases who had not received a test but were assumed negative based on prevalence figures, and to those who had received a negative test. For the informal private sector across three endemic countries - Kenya, Uganda and Tanzania - irrational ACT use was estimated at approximately 10 million. At 64 cents a course, approximately $6.8 million worth of ACTs are being wasted on those without malaria. Given that this represents only small a subset of ACT misuse across sub-Saharan Africa, it is important that mRDT scale-up continues, especially in the informal private sector where a third of all treatment-seeking occurs. Further, drug shop owners must be properly trained on mRDT use and should be empowered to trust mRDT results in diagnosing malaria.

1526

EFFICACY OF DIHYDROARTEMISININ-PIPERAQUIN AND CHLOROQUINE IN THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM AND PLASMODIUM VIVAX* MALARIA IN VIETNAM

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Resistance to antimalarial drugs is a major public health problem, which may hamper the control of malaria. In order to deal with the growing resistance of both *Plasmodium falciparum* and *P. vivax*, surveillance of the first choice antimalarial drugs- DHA-PPQ and chloroquine, was conducted in the Central Vietnam during 2012 to 2015. The surveillance was conducted at 3 sentinel sites in Quang Tri, Gia Lai and Ninh Thuận based on the World Health Organization protocol (WHO, 2009). Standard total dosage of DHA-PPQ 40/320 mg (8 tablets, in 3 days, chloroquine 500 mg (10 tablets in 3 days) for adult patients, with 28 or 42 day follow-up. K13 gene mutation identification was also done at the Institute Pasteur of Phnom- Penh (2014) and the Department of Microbiology, University of Sassari, Italy (2015). During the period from 2012 -2015 the DHA-PPQ efficacy to *P. falciparum* malaria were high with APCR, (69/69, 100%) (46/46, 100%) in Quang Tri and Ninh Thuan province respectively, but APCR (57/60, 95%), ETF (2/60; 3.33%), LCF (1/60, 1.67%) in Gia Lai sentinel site. The mean parasite clearance time (PCT) was 48 hours except 11 cases in Gia Lai having blood smear positive rate on D3 of 18.3%. In those cases there were 9 mutations in Kelch 13 propeller gene (C580Y, R539T). The efficacy of CQ on the clearance of blood stage *P. vivax* (without primaquine) was still high with ACPR at 100% in Quang Tri province, Gia Lai province, in Ninh Thuan province. There was no recurrence with 28 days follow-up. Conclusions: DHA-PPQ and chloroquine remain efficacious for the treatment of uncomplicated falciparum and vivax malaria respectively in the Central of Vietnam. Further investigation as detection of artemisinin resistant markers and PK/PD are needed.

1527

PHARMACOKINETIC INTERACTIONS OF ARTESUNATE ON THE DISPOSITION OF AMODIAQUINE IN SUBJECTS WITH *PLASMODIUM FALCIPARUM* INFECTION AFTER ORAL ADMINISTRATION OF FIXED-DOSE COMBINATION OF AMODIAQUINE-ARTESUNATE

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Artemisinin-based combination therapy has been adopted by several African countries including Nigeria as first line treatment for uncomplicated falciparum malaria. There is a need to balance the

advantages of the combination against the possible effect of interaction between the component drugs. We investigated the pharmacokinetic interactions of artesunate (AS) on the disposition of amodiaquine (AQ) in subjects with *Plasmodium falciparum* infection after oral administration of fixed-dose combination of amodiaquine-artesunate (AQAS). This is a randomized, open-label trial in which twenty subjects with *P. falciparum* infection were assigned into two treatment arms namely, AQ or in combination with artesunate (AQ/AS). AQ (600mg) or a fixed dose combination of AQ/AS (AQ 306.3 mg/AS 100 mg × 2 tablets) was administered once daily for 3 days. Blood samples were collected at different sampling times. Subjects were followed up for 28 days to assess response to treatment; those who failed to respond to AQ or AQ/AS were treated with artemether/lumefantrine and quinine respectively. Plasma was obtained and assayed for AQ and desethylamodiaquine (DAQ) levels using hplc technique. The pharmacokinetics parameters of AQ and DAQ were determined and compared in the two arms. There are no statistically significant difference in the peak plasma concentration, Cmax (774.34 ± 146.94 vs. 763.19 ± 89.99 ng/ml), concentration on day 7, Conc day 7 (357.13 ± 45.06 vs. 390.88 ± 53.63 ng/ml), total drug exposure, AUC0-∞ (187,710 ± 14.110 vs. 197,960 ± 14, 6874 ngh/ml) and elimination half-life, T½ (212.81 ± 1.24 vs. 212.89 ± 1.20 h) (P >0.05) of DAQ in AQAS vs. AQ respectively. The pharmacokinetic parameters of AQ were also similar in both arms (P > 0.05). Parasites cleared in all subjects in the two arms except in a subject in AQ arm in whom parasites were seen on Day 14. Although not significant, the reduced total exposure of AQ in AQAS arm was a concern particularly in areas with reduced AQ sensitivity. Further studies are needed to assess the degree of reduction in total exposure of DAQ observed in this study so as to design optimal dosing/tolerability profile for AQ use.

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UNEXPECTED FALL IN HEMOGLOBIN VALUE DURING THE FIRST PHASE OF MALARIA PREVENTION TRIAL: PRELIMINARY FINDINGS FROM A DROUGHT PRONE AREA IN ETHIOPIA

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As part of a field trial to provide evidence on the combined use of LLINs and IRS for malaria prevention, we measured hemoglobin values among children less than five years old. Our hypothesis was that by preventing malaria, the mean hemoglobin values would increase by 0.5 gm/ dl. In this trial we followed about 3000 children 6 to 59 months old from August 2014 to December 2015. We did active (weekly home visit) and passive malaria case search. Hemoglobin (HB) concentration surveys were conducted after the major malaria transmission seasons in 2014 and 2015, and children with HB < 11 gm/dl were classified as having anemia. The mean HB value decreased from 11.6 gm/dl in 2014 to 11.2 g/dl in 2015 (mean difference 0.35 gm/dl (95% CI 0.27 - 0.43; P<0.001). In 2014, the prevalence of anemia was 28.2% (95 % CI; 26.6 - 29.8) and increased in 2015 to 36.8% (95 % CI; 35.1 - 38.5). Among 171 registered malaria cases, 88 (51.5%) were due to *Plasmodium falciparum*. Among these children, malaria incidence rate was 8.6 (95% CI; 6.5 - 11.3) in 2014, and 8.3 (95% CI 6.4 - 10.8) cases per 10,000 person weeks in 2015. The mean hemoglobin value as well as malaria incidence increased with increasing age of the child. Family wealth and educational status of the head of households were predictors of anemia, but malaria incidence was not associated with anemia. This study showed an unexpected fall in mean HB value between the two surveys. This occurred in spite of malaria prevention efforts. During the same period, the region experienced one of the most severe droughts in decades. Even if many children in the study area received supplementary feeding, and we believe the worsening food household insecurity may explain the increase in anaemia prevalence. This study demonstrated that doing field trials in drought prone areas may bring unexpected challenges.

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SAFETY AND TOLERABILITY OF ROSIGLITAZONE ADJUNCTIVE THERAPY FOR CHILDREN WITH UNCOMPLICATED MALARIA: A RANDOMIZED, DOUBLE BLIND, PLACEBO CONTROLLED TRIAL

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Studies have shown that the oral antidiabetic Rosiglitazone can improve malaria outcomes in adults, by decreasing the levels of Angiopoietin II (AGII), an independent and quantitative biomarker of disease severity in malaria. We present the first results of a pilot clinical trial assessing the safety and efficacy of rosiglitazone as adjunctive therapy for *Plasmodium falciparum* malaria in Mozambican children. Thirty children (1-12 years) with uncomplicated malaria were randomized (2:1) to receiverosiglitazone (0.045mg/kg/dose) or placebo (double blind) twice-daily for four days. ECG, Blood glucose levels, biochemical and haematological parameters were monitored for safety. AGII and other biomarkers of host response including endothelial activation, inflammation, coagulopathy, and neuroprotection were measured for efficacy. Results: No significant differences were found in terms of the incidence of biochemical, haematological or electrocardiographic abnormalities. Efficacy results will be presented. In conclusion, this study confirmed the safety of Rosiglitazone in Mozambican children with malaria. Evaluation of Rosiglitazone as adjuvant therapy for severe malaria is warranted.

1530

SAFETY AND EFFICACY OF REPEATED ADMINISTRATION OF PYRONARIDINE-ARTESUNATE OR DIHYDROARTEMISININ-PIPERAQUINE VS ARTESUNATE-AMODIAQUINE IN CHILDREN AND ADULT PATIENTS WITH ACUTE UNCOMPLICATED *PLASMODIUM* SP MALARIA OVER OF TWO YEARS PERIOD AT BANFORA/NIANGOLOKO SITE IN BURKINA FASO

Issiaka Soulama, Aboubacar Sam Coulibaly, Moise J. Kaboré, Maurice Ouattara, Edith C. Bougouma, Souleymane Sanon, Noélie Henry, Amidou Diarra, Daouda Ouattara, Amidou Ouedraogo, Alphonse Ouedraogo, Benjamin Sombie, Alfred B. Tiono, Sodiomon B. Sirima

Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso

The safety and efficacy of repeated administration of three ACTs [(pyronaridine-artesunate (PYR) or dihydroartemisinin-piperaquine (DHA-PQ) vs artesunate-amodiaquine (ASAQ)] were evaluated in West African Countries, members of the West African Network of Clinical trials for AntiMalarial drugs (WANECAM). In the current study we present the preliminaries data of repeated administration of PYR or DHA-PQ vs ASAQ over a period of 2 year in children and adults with uncomplicated Plasmodium sp malaria at Banfora/Niangoloko sites in Burkina Faso. This study is a comparative, randomized, open label longitudinal clinical trial involving children and adults with uncomplicated *Plasmodium* sp. malaria. Each of participant enrolled received during their subsequent episodes the same drug and went through the same trial procedures as for the initial episode. A total of 1090 participants were screened from which, 763 were enrolled in ASAQ (315), DHA-PQ (224) and PYR (224) arm, from July 2012 to December 2013. As per age 342, 357 and 64 participants aged < 5 years, 5-14 years and ≥ 15 years were followed respectively during

the two years. The preliminaries results showed that 245 of 315 (77.8%) patients, 166 of 224 (74.10%); and 176 of 224 (78.6%) experienced at least 2 malaria episodes and 108 (34.3%), 63 (28.1%) and 82 (36.6%) experienced at least 5 malaria episodes in the ASAQ, DHA-PQ and PYR arms respectively. The average time between the first and the second malaria episode was statistically longer ($p<0.05$) in DHA (157 days) compared to ASAQ (135 days) and PYR (117 days) arms. Adequate clinical and parasitological response (ACPR) by day 28 was 93.0 %, 97.8% and 98.2% in ASAQ, DHA and PYR arm respectively during the first malaria episodes. The 42 day cure rate (not adjusted by PCR) was 80.3 %, 93.8% and 78.2% in ASAQ, DHA-PQ and PYR arms respectively during the first malaria episodes. Our preliminary results confirmed the two news drugs (DHA-PQ and PYR) are safe and their efficacy comparable to the ASAQ in uncomplicated malaria treatment in high malaria transmission region.

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RANDOMIZED, BLINDED CLINICAL TRIAL COMPARING AN INVESTIGATIONAL ANTIMALARIAL, AQ-13, TO ARTÉMETHER + LUMÉFANTRINE FOR TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

Ousmane A. Koita[1], Lansana Sangare[1], Haiyan Deng[2], Sahare Fongoro[1], Moctar Coulibaly[1], Aliou Sissako[1], Trevor A. Thompson[2], Youssouf Diarra[1], Mamadou Ba[1], Ababacar Maiga[1], Boubakar Diallo[1], Frances J. Mather[2], Asif Anwar[2], Donald J. Krogstad[2]

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Chloroquine (CQ) was the treatment of choice for *Plasmodium falciparum* malaria until CQ-resistant parasites were identified in Southeast Asia and South America over 50 years ago. Although CQ-resistant *P. falciparum* have been an obstacle to malaria control since that time, recent studies have shown 4-aminoquinolines (4-AQs) with modified side chains are active against CQ-resistant parasites and that the lead compound (AQ-13) is safe in human subjects. To determine whether AQ-13 is effective for the treatment of uncomplicated malaria, we performed a randomized, blinded clinical trial in which 66 Malian men received oral treatment with AQ-13 or the current recommendation of artémether + luméfantrine (A+L) for 3 days. After all subjects completed the 42 day follow-up, the study was unblinded and results were compared for subjects randomized to A+L vs. AQ-13. There were no differences in the ages, initial parasite counts or Hb levels of subjects randomized to A+L vs. AQ-13. Likewise, High Resolution Melt (HRM) analysIs indicated that similar numbers of subjects with CQ-susceptible, CQ-resistant and mixtures of -susceptible and -resistant *P. falciparum* were randomized to A+L and AQ-13. Subjects treated with either A+L or AQ-13 cleared asexual parasites from their blood on or before day 7 and no serious, Grade 3 or Grade 4 AEs occurred in either group. There were 2 withdrawals for personal reasons from the AQ-13 arm on day 4 after asexual parasites had been cleared on day 3 and 2 recurrences in the A+L arm on follow-up days 17 and 21. These results indicate AQ-13 is efficacious and safe for the treatment of uncomplicated malaria caused by CQ-susceptible and –resistant *P. falciparum*. Based on this study, the efficacy of AQ-13 cannot be distinguished from the efficacy of A+L for uncomplicated *P. falciparum* malaria. Efficacies for A+L and AQ-13 were 94% (31/33) based on intent to treat and 94% and 100% (31/33 and 31/31) based on per protocol analyses.

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RANDOMIZED, BLINDED CLINICAL TRIAL COMPARING AN INVESTIGATIONAL ANTIMALARIAL, AQ-13, TO ARTÉMETHER + LUMÉFANTRINE FOR TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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A TRIAL OF SEASONAL MALARIA CHEMOPREVENTION PLUS AZITHROMYCIN IN AFRICAN CHILDREN

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Mass administration of azithromycin (AZ) on a single occasion for elimination of trachoma in Ethiopia was associated with a substantial reduction in overall child mortality in communities treated with AZ compared to untreated control communities. This reduction was sustained for 26 months after treatment (Rate Ratio 0.35; 95% C1: 0.17, 0.74). The impact of AZ on mortality could have been achieved through its action on pneumococci and other bacteria. As seasonal malaria chemoprevention (SMC) is implemented in countries of the Sahel and sub-Sahel, it would

be possible to add AZ to the antimalarial drug regimen given during the rainy season when the risk of both malaria and severe bacterial infections is highest. Thus, we are conducting a study in Mali and Burkina Faso to determine whether addition of AZ to SMC using sulphadoxine/pyrimethamine (SP)+amodiaquine (AQ) provides an additional reduction in deaths and severe illness in young African children. This is a double blind, randomised, placebo-controlled trial involving 19200 children aged 3 - 59 months who are randomised by household to receive four rounds of either SP+AQ+AZ or SP+AQ+ placebo at monthly intervals during the peak malaria transmission season over a three-year period. Administration of the first round of drugs started in August 2014 and the final round will be completed in November 2016. In 2014 and 2015, the proportion of children who received ≥3 monthly rounds of drug each year was >90%. We will present the study rationale and design, and preliminary results of blinded analysis of the incidence of primary (hospital admission or death during the transmission season) and selected secondary endpoints (incidence of clinical malaria, respiratory infection and diarrhoea, and the prevalence of malaria parasitaemia, markers of SP resistance, malnutrition and pneumococcal carriage at cross-sectional surveys). Final results will be available in 2017.

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A PROOF-OF-CONCEPT, RANDOMIZED STUDY IN NON-IMMUNE HEALTHY ADULT VOLUNTEERS TO INVESTIGATE THE SAFETY, TOLERABILITY, PHARMACOKINETIC PROFILE AND PROPHYLACTIC ACTIVITY OF A SINGLE DOSE OF DSM265 IN A CONTROLLED HUMAN MALARIAL INFECTION CHALLENGE EITHER BY DIRECT VENOUS INOCULATION OF *PLASMODIUM FALCIPARUM* SPOROZOITES (PFSPZ) OR A SINGLE EPISODE OF BITES BY MOSQUITOES CARRYING *P. FALCIPARUM*

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In the face of rising drug resistance, new anti-malarial drugs are needed for prophylaxis and radical cure. DSM265 is a novel triazolopyrimidine-based inhibitor of dihydroorotate dehydrogenase (DHODH), a key enzyme in the pyrimidine biosynthesis pathway. DSM265 demonstrated promising *in vitro* and *in vivo* activities against liver and blood stages in preclinical studies and recently advanced to clinical trials for blood stage infection. In collaboration with the Medicines for Malaria Venture and the University of Tübingen, the Seattle Malaria Clinical Trials Center initiated a study to assess the safety, tolerability, pharmacokinetic profile and prophylactic activity of a single dose of DSM265 in a controlled human malarial infection (CHMI) challenge either by direct venous inoculation (DVI) of *Plasmodium falciparum* sporozoites (PfSPZ) or a single episode of bites by mosquitoes carrying *P. falciparum*. This study is designed to determine the initial dosing interval for once-weekly chemoprevention. Three cohorts (n=6 drug-treated plus n=2 placebo controls) are planned to assess DSM265 dosing 3 or 7 days prior to PfSPZ DVI CHMI or 7 days prior to mosquito bite CHMI. Subjects will be followed using the standard CHMI model using a *Plasmodium* 18S rRNA molecular qRT-PCR-based treatment threshold to initiate rescue treatment after ≥250 estimated parasites/mL are detected. Pharmacokinetic data on DSM265, safety laboratory data, adverse events and parasite growth kinetics will be assessed. The study is ongoing, with data from completed cohorts to be presented.

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PHASE II STUDY OF ARTEFENOMEL (OZ439) AND PIPERAQUINE TO INVESTIGATE SINGLE DOSE TREATMENT FOR UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA

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We performed a clinical phase II study with single dose combinations of artefenomel (OZ439) and piperaquine phosphate (PQP) at three dose levels as part of the development of a single dose cure for uncomplicated *Plasmodium falciparum* malaria to improve patient compliance, reduce risk of drug resistance, and to support eradication campaigns. Patients (n=448) in Africa (n=365) and Asia (n=83) were recruited with 85% being children <5 yrs of age. OZ439/PQP combination demonstrated an acceptable safety profile and was well tolerated. The primary endpoint was Day 28 PCR-adjusted ACPR (ACPR28). ACPR28 for the combination of 800mg OZ439 with 640 mg PQP, 960 mg PQP and 1440 mg PQP was 68.4%, 70.8% and 78.6% respectively, in per protocol population. ACPR28 was lower in Asian than African patients despite achieving higher drug exposures. Only 1 early treatment failure occurred. The success of a single dose treatment is assumed to be dependent on adequate parasiticidal drug exposure for three parasite life-cycles (approximately 1 week). Population PK analyses using non-linear mixed effect modelling allowed estimation of concentration at Day 7 and identification of influential covariates in this study. The probability of achieving ACPR28 was a function of both OZ439 and PQP concentrations at Day 7, as well as baseline parasitemia, and region. Neither age nor presumed immunity was identified as a covariate. In Asia, >70% patients had parasites with mutations in the Kelch13 (K13) propeller gene. K13 genotype, known to alter parasite reduction rate for artesunate also impacted the parasite clearance by OZ439/PQP but had no significant impact on the cure rate measured as ACPR28. Furthermore, K13 genotype was not a significant covariate in the model for ACPR28. In conclusion, none of the treatment arms investigated in this study met the regulatory efficacy threshold defined as ACPR28 >95%. Given that there are increasing reports of PQP resistance in South-East Asia, we decided not to follow up on the potential of this combination for a 3-day regimen, instead we plan to investigate combinations with newer compounds where there is less risk of pre-existing resistance.

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TOLERABILITY OF SINGLE DOSE PRIMAQUINE IN G6PD-DEFICIENT ADULT MALES IN MALI WITHOUT MALARIA: AN OPEN-LABEL PHASE 2 DOSE-ADJUSTMENT TRIAL

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Single low dose primaquine (SLD-PQ) is recommended by the WHO to block the transmission of *Plasmodium falciparum* parasites to mosquitoes. However, uptake is limited due to concerns of hemolytic side effects in individuals with enzymatic glucose-6-phosphate dehydrogenase (G6PD) deficiency. We determined the safety of three, single-dose regimens of primaquine in G6PD-deficient adult males without malaria in Mali. We conducted an open-label, non-randomized, dose-adjustment trial of the tolerability of 0.40, 0.45, and 0.50 mg/kg of single dose primaquine in G6PD-deficient adult males in Mali without malaria. Adult males with a Carestart® qualitative G6PD-deficient test result were treated with a

0.40, 0.45, or 0.50 mg/kg dose of primaquine, followed by a 0.50 mg/kg control group of G6PD-replete men. The primary outcome was the within-person percentage change in hemoglobin concentration, assessed using a Hemocue system, from baseline levels between day 0 and day 10. All individuals who received a single-dose of primaquine and completed safety assessments--comprising hemoglobin concentration, urine color, and clinical assessment--daily on days 1-10, and on days 14 and 28 following primaquine administration, were included in the primary sample analysis (n=28). We enrolled 28 participants sequentially, from August 13 to December 19, 2015. Primaquine doses of 0.40, 0.45 and 0.5 mg/kg were all found to be safe and tolerable. A hemolytic dose response was not observed at these doses in any of the participants, no serious adverse events were reported, and adverse events were not associated with the treatment group. SLD-PQ up to 0.50 mg/kg was well tolerated in healthy G6PD-deficient populations in West Africa, and should be rolled out using 0.50 mg/kg as the upper bound for weight-based dosing bands.

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THE ETHICS OF USING A PLACEBO ARM IN RANDOMIZED CONTROLLED TRIALS: A CASE OF IN A PRIMAQUINE ANTIRELAPSE STUDY

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Clinical research involving randomised controlled trials is critical for advancing global health. However global health trials can evoke important ethical issues, particularly the use of placebo or non-intervention control arms. The issue has generated ethical consideration for many years, that hinges on the debate on "double standard of care". We explore this through evaluation of the use of a placebo arm in the specific example of a large multicentred placebo-controlled, double-blinded, randomized trial to determine primaquine antirelapse efficacy in vivax malaria. The trial involves almost 2000 patients enrolled in Indonesia, Vietnam, Ethiopia, Pakistan and Afghanistan. There are three arms - chloroquine or an artemisinin combination therapy treatment plus either: 7 days primaquine , 14 days primaquine or 14 days placebo. The need for the study is justified in view of the poor evidence for the current WHO recommended regimen of 14 days primaquine. The ethical rationale for including a 14-day placebo arm can be made on the grounds that the standard of care in most endemic countries does not include in reality widespread, routine use of primaquine. We argue that since there is equipoise among the study arms, the risk of being in the placebo arm is no greater than the risk of not being in the trial and that there are no double standards. This analysis complements others in literature with regards to the use of placebo or no intervention treatment arms, and highlights that such debate case be justified on its own merits rather than relying on general guidelines.

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INHALED NITRIC OXIDE IMPROVES NEUROCOGNITIVE OUTCOMES IN CHILDREN WITH SEVERE MALARIA AND LACTIC ACIDOSIS

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Severe malaria is a leading cause of neurocognitive impairment in African children. Low levels of bioavailable nitric oxide (NO) are associated with severe malaria. Supplementation with inhaled NO (iNO) has been shown to be neuroprotective in term or near-term infants with persistent pulmonary hypertension but the neuroprotective actions of iNO have not been documented in systemic infections. This randomized, double-blind, placebo-controlled trial compared the effect of inhaled nitric oxide (iNO) at 80ppm versus room air (placebo) on neurocognitive function in children with severe malaria treated with parenteral artesunate. Children aged 1 to 10 years received either iNO or placebo. Six months post-discharge, neurocognitive testing was performed to assess overall cognition, attention, associative memory, executive function, motor skills, language and visual reception. We compared test scores between the treatment arms and the frequency of impairment in all domains between the arms. At six months, 61 children in the iNO arm and 59 children in the placebo arm were evaluated. 35% of children had impairment (defined by a z-score ≤-2SD) in at least one domain. There were no significant differences in z-scores for overall cognition, attention, associative memory and executive function between iNO and the placebo group. Children receiving iNO were less likely to have multiple impaired domains (11.5% vs 25.4%, p=0.048) and to have fine motor impairment, relative to children receiving placebo (8.2% vs 22.0%, p=0.034). Subgroup analysis in children with acidosis at admission showed iNO was associated with improved attention (p=0.001), fine motor functioning (p=0.004), visual reception (p=0.028), receptive language (p=0.015), and overall cognitive function (p=0.009). Inhaled nitric oxide is associated with better cognitive outcomes in children with severe malaria presenting with acidosis.

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A RANDOMIZED TRIAL OF THE SAFETY OF LOW DOSE PRIMAQUINE IN THE TREATMENT OF G6PD NORMAL AND DEFICIENT ADULT PATIENTS WITH *PLASMODIUM FALCIPARUM* MALARIA IN SENEGAL

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WHO recommends the addition of a single dose of primaquine (0.25 mg base/kg) to artemisinin combination treatments (ACTs) as a component of pre-elimination or elimination programs. However, primaquine has been little used in Africa and there are concerns about its safety, as the drug can cause acute haemolytic anaemia in individuals with G6PD deficiency. This open randomised controlled trial was conducted to assess the safety of adding low-dose primaquine to the normal ACT regimen (AL, ASAQ, DHAP) in adult patients in Senegal. Patients with *P. falciparum* malaria (parasitaemia {1,000-100,000} trophozoites/µL) were randomized to receive treatment with ACT or ACT plus low-dose primaquine. Haemoglobin concentration was measured at enrolment, and on days 3, 7, 14, 21 and 28 post-treatment. G6PD status was determined for each patient using a qualitative field test (CareStart™). The primary

outcome was the change in haemoglobin concentration from day 0 to day 7, which was compared between trial arms using analysis of covariance. Secondary endpoints included haemoglobin variation from day 0 to day 28. Two hundred and seventy five patients (137 in the ACT arm and 138 in ACT plus primaquine arm) were randomized. At enrolment, gender, mean weight, parasitaemia, haemoglobin, and prevalence of G6PD deficiency were similar in the two arms. Mean haemoglobin concentration on day 7, was similar in primaquine and control groups (11.9 and 12.1 g/dL respectively). The difference in Hb concentration on day 7 in the primaquine group compared to controls after adjusting for Hb at baseline was -0.029 (95%CI -0.51,0.45) g/dL. Haemoglobin change at day 7 was significantly associated with haemoglobin at enrolment, weight and gender. There was no evidence of an association with treatment drug, G6PD status, and parasiteamia at enrolment. Haemoglobin concentrations recovered and exceeded baseline level by day 28. The administration of single low dose primaquine (0.25 mg/kg) in addition to ACT treatment, to adult patients with acute *P. falciparum* malaria, is safe and does not induced significant drop in haemoglobin level both for G6PD normal and deficient individuals.

1540

MODERATE AND SEVERE LFT ELEVATIONS IN CONTROLLED HUMAN *P. FALCIPARUM* MALARIA INFECTION MODEL: RECENT EXPERIENCE, LITERATURE REVIEW AND MECHANISTIC HYPOTHESES

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Controlled Human Malaria Infection (CHMI) in healthy subjects is a critical model in vaccine research and in profiling new chemical entities (NCEs). Documented laboratory changes induced by CHMI include mild benign liver function tests (LFT) elevations (<2.5xULN). In recent evaluations of antimalarial NCEs with the *Plasmodium falciparum* (P.f.) induced blood stage malaria (IBSM) model, we have observed cases of transient asymptomatic moderate/severe LFT elevations (2.6-10xULN). Among more than 174 healthy subjects tested in our P.f. IBSM studies with NCEs and approved antimalarials, 6 participants showed ALT/AST elevations of up to 10 x ULN. These cases were reported in two distinct studies (3/8 subjects each) with two NCEs that were not identified as hepatotoxic in the initial Phase 1 studies. A liver safety review of 13 completed P.f. IBSM studies (7 approved antimalarials and 6 NCEs) and 44 published sporozoite challenge studies was performed. For sporozoite CHMIs, moderate/severe LFT elevations were also reported in a mosquito-bite study with a NCE (pafuramidine) in 6/19 subjects (4 active/2 placebo). Most of these subjects (including placebo) received acetaminophen with a highest cumulative dose of 17.5g. ALT elevations were generally higher than AST. For IBSM studies, only one subject showed bilirubin > 2xULN (potential Hy´s law reported as serious adverse event). Review of these cases by Drug-Induced Liver Injury Experts suggest that these changes are likely to be multifactorial in origin with combined interaction of 3 possible causative factors: 1- Inflammatory state induced by CHMI, 2-Acetaminophen, 3-NCE and additional risk factors (undiagnosed condition such as liver steatosis or alcohol consumption). Because these laboratory findings are not uncommon, specific safety provisions for the conduct of CHMI studies with NCEs during drug development are proposed. The recommendations for IBSM studies include preclinical hepatotoxicity profiling of the NCE, strengthened eligibility criteria, use of a positive control and symptomatic treatment with NSAIDs (ibuprofen) as a substitute to acetaminophen.

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HARMONIZATION OF A MULTI-CENTER ARTESUNATE-MEFLOQUINE DOD CLINICAL EFFICACY STUDY FOR PATIENTS WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN KENYA, PERU AND THAILAND

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Artemisinin-resistant *Plasmodium falciparum* is of growing concern in Southeast Asia, with potential to spread to other *P. falciparum* endemic regions. To conduct surveillance for emerging artemisinin resistance in geographically distinct regions, the AFHSB-GEIS leveraged DoD laboratories in Kenya, Peru, and Thailand to coordinate a multi-center harmonized trial for artesunate-mefloquine efficacy, based on parasite clearance rates for 72 hours after artesunate dosing, using a standardized approach based on WHO efficacy study methodology. Here we describe specific activities designed to improve synchronization and data comparability across study sites, as well as challenges and lessons learned. Participating DoD organizations jointly developed clinical and laboratory quality assurance programs and plans, study documents and case report forms, and a reciprocal monitoring strategy to facilitate harmonization across sites. Each site conducted self-monitoring and reciprocal monitoring of other sites' progress for adherence to the clinical protocol and quality assurance plans. Microscopy proficiency testing was conducted at the initiation and evaluated at multiple points throughout the study. Data management was centralized among sites to ensure data integrity, including the reception of raw data, cleaning, querying, and producing complete datasets for analysis. Site personnel were trained in correctly populating clinical data from site-specific case report forms into harmonized case report forms for submission to a clinical data management system used to provide quality control. Challenges incurred included the removal of a participating site, protocol enrollment following evolving *P. falciparum* transmission patterns, and data submission technical difficulties. Future coordinated efforts will be directed at harmonized *in vitro* drug efficacy testing and genetic studies for markers of artemisinin resistance.

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DETERMINATION OF CYTOCHROME P-450 ISOENZYME 2D6 (CYP2D6) GENOTYPES IN AN ACTIVE-DUTY U.S. MILITARY POPULATION AND THE PHARMACOGENOMIC IMPACT ON PRIMAQUINE METABOLISM

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Plasmodium vivax malaria is a leading cause of malaria in U.S. service members and radical cure therapy requires a 2-week course of primaquine (PQ) to eliminate the latent hypnozoites and prevent relapse infection. Cytochrome P450 isoenzyme 2D6 (CYP2D6) is a key enzyme involved in converting parent PQ into the active anti-malaria metabolite. Allelic polymorphisms determine CYP2D6 genotype, and these differences result in variations in enzyme activity or phenotype. CYP2D6 phenotypes are assigned based on expected impact of enzyme activity on drug metabolism: no activity (Poor Metabolizers (PM)), decreased activity (Intermediate Metabolizers (IM)), normal activity (Extensive Metabolizers (EM)), and increased activity (Ultra rapid Metabolizers (UM)). Given the risk of *P. vivax* infection in U.S. Service members deployed to endemic

areas, we sought to characterize the prevalence of CYP2D6 genotypes and associated phenotypes of the enzyme in this population. Approximately 450 active duty personnel underwent CYP2D6 genotyping by a multiplexed cytometric bead array assay, Luminex xTAG® CYP2D6 Kit v3 (Austin, TX) allowing for detection of the major alleles in the United States: 1,2,3,4,5,6,7,8,9,10,11,15, 17,29, 35,41. A subset of volunteers were administered a one-time oral dose of 30 mg PQ. Blood and urine were collected at various timepoints in order to measure concentrations of PQ, carboxyprimaquine, and phenolic metabolites by ultra-performance liquid chromatography with mass spectrometry (UPLC-MS) and determine the pharmacokinetic (PK) parameters. The results of CYP2D6 genotypes and the effect of CYP2D6 phenotype on PQ metabolism will be presented. Results from this study will inform both DoD force health protection policy for the use of primaquine and future prospective *in vivo* studies of anti-relapse treatment of *P. vivax* infection.

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THE ROLE OF PRIVATE HEALTH CARE PROVIDERS IN ACHIEVING MALARIA ELIMINATION IN ACEH BESAR DISTRICT

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Malaria elimination is a goal of the Government of Indonesia. The District of Aceh Besar has promulgated Regent's Regulation No. 23/2013 which formally commits to achieve elimination by 2015. However, the role of private health care providers in progressing towards malaria elimination has not been identified. A survey of six types of private health care providers through a simple random sampling method has been conducted. Primary and secondary data were collected from 153 providers from March to August 2014 on occupational characteristics, availability of malaria-related supplies, knowledge of malaria, and involvement in the malaria elimination program. Data analysis was done using Chi-Square test and logistic regression with EPI Info version 7. The result showed that educational background (p=0,045), participation in malaria training (p=0,004), occupational characteristics (p=0,004) and knowledge of malaria (p<0,001) were associated with involvement in malaria elimination program. Additionally, roles of private health care providers in malaria elimination were predominantly influenced by having good knowledge of malaria (OR 8.1; 95% CI 3.8-17.5) and participation in malaria training (OR 2.7; 95% CI 1.2-6.3). The contribution private providers to officially reported data for 2013 showed that, 13.5% of suspected malaria cases were laboratory-confirmed, 7.4% of malaria cases were treated by ACT, and 5.6% malaria cases treated were reported to government. At pharmacies and drug stores, an average of 4 people sought medication for malaria daily, with pharmacies selling a mean of 38 Chloroquine tablets monthly. Private health care providers play a pivotal role in diagnosis, treatment, prevention and recording-reporting of malaria in area moving toward malaria elimination. The private providers in Aceh Besar falls far short of standards for diagnosis, treatment and reporting set by the public section. This highlights the need for established of an effective public-private network to ensure adherence to standards, effective monitoring, and good communication.

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VILLAGE MALARIA WORKER PERFORMANCE KEY TO THE ELIMINATION OF ARTEMISININ-RESISTANT MALARIA: A WESTERN CAMBODIA HEALTH SYSTEM ASSESSMENT

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Village Malaria Workers (VMWs) and Mobile Malaria Workers (MMWs) are a critical component of Cambodia's national strategy to eliminate *Plasmodium falciparum* malaria by 2025. Since 2004, VMWs have been providing malaria diagnosis through the use of Rapid Diagnostic Tests (RDTs), and free-of-charge Artemisinin-based Combination Therapies (ACTs) in villages more than 5 kilometres away from the closest health facility. This study aimed to assess the job performance of VMWs/MMWs, and identify challenges they face, which may impede elimination efforts. *Methods.* A mixed-methods assessment was conducted in five provinces of western Cambodia. 185 VMW/MMW participants were surveyed using a structured questionnaire. Qualitative data was gathered through a total of 60 Focus Group Discussions (FGDs) and 65 In-depth Interviews (IDIs). Data triangulation of the qualitative and quantitative data was used during analysis. Overall, VMWs/MMWs met or exceeded the expected performance levels (80%). Nevertheless, some performance gaps were identified. Misconceptions regarding malaria transmission and prevention were found among workers. The recommended approach for malaria treatment, Directly Observed Treatment (DOT), had low implementation rates. Stock outs, difficulties in reaching out to Migrant and Mobile Populations (MMPs), insufficient means of transportation and dwindling worker satisfaction also affected job performance. VMW/MMW job performance must be increased from 80% to 100% in order to achieve elimination. In order to do this, it is recommended for the national malaria program to eliminate worker malaria knowledge gaps. Barriers to DOT implementation and health system failures also need to be addressed. The VMW programme should be expanded on several fronts in order to tackle remaining performance gaps. Findings from this evaluation are useful to inform the planning of future activities of the programme and to improve the effectiveness of interventions in a context where artemisinin drug resistance is a significant public health issue.

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DESIGNING MALARIA ELIMINATION STRATEGIES TO ACHIEVE HIGH COMMUNITY UPTAKE: FINDINGS FROM A FORMATIVE RESEARCH STUDY IN THE DEPARTMENT OF GRAND ANSE, HAITI

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The success of malaria elimination efforts depends on high community acceptance and uptake of effective interventions. This is particularly challenging in low transmission settings, such as Haiti, where malaria is one of many competing health issues. Haiti has taken on the ambitious goal of eliminating malaria by 2020. To do so, aggressive strategies such as targeted mass drug administration (MDA) will likely be used. We conducted formative research to inform the design of elimination strategies using qualitative methods. Key informant interviews (n=6), in-depth interviews (n=9), and focus group discussions (n=7) were conducted from December 7-19 of 2015 with purposefully selected health agents, traditional healers, community leaders, and community members. Data were transcribed, coded and analyzed in relation to emergent themes. Findings indicate that elimination strategies should include multiple actors from the Haitian pluralistic health system. Results from social influence mapping suggest formal healthcare providers, as well as 'Hougans' [voodoo priests], would influence community uptake of interventions. Incentives and disincentives for both groups related to the goal of malaria elimination, and for working collaboratively, should be addressed. It is important to leverage community resources including community leaders (teachers, priests) and organizations (churches, social aid clubs, schools) so that positive messaging is reinforced across multiple sources, and uptake is modeled by appropriate social influences. This is especially important for harder-to-reach populations including youth and men. The intervention should prospectively target misinformation and rumors that may develop concurrent with roll-out. Uptake may benefit from ongoing monitoring of community perceptions during implementation of elimination strategies, and coupling traditional social marketing and communication techniques with social network strategies. Results from Haiti will likely have broader implications for other low transmission settings aiming to eliminate malaria.

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TARGETED COMMUNITY SENSITIZATION TO REDUCE ANTICIPATED REFUSALS IN MALARIA MASS DRUG ADMINISTRATION TRIAL: LESSONS LEARNED FROM SOUTHERN ZAMBIA

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The Zambia National Malaria Control Programme has embarked on an ambitious goal of eliminating malaria in Southern Province of Zambia and nationally by 2020. One component to accelerate toward the elimination goal includes selective use of mass drug administration (MDA) with dihydroartemisinin piperaquine (DHAp). As part of a recent trial to evaluate the effectiveness of MDA, 20 randomized health facility catchment areas were wholly targeted for this community based treatment strategy. In anticipation of research-based field activities, several community sensitization activities were conducted to promote the uptake of MDA at community, household, and individual levels in 2014 and 2015. High coverage was deemed essential to the effectiveness of MDA and refusals were monitored closely through household surveys carried out during treatment campaigns associated with the trial interventions. Brochures and job aids were pretested and targeted to community members and local leaders who might influence trial participation and treatment adherence. Radio scripts were developed, translated into the local language, and aired on local community radio stations as a program with recognizable jingles. Community entry meetings scheduled through the chief's palace ensured community members could hear directly from their local MOH staff about the study, have their questions answered, and enjoy drama group performances which emphasized key messages with the audience. Campaign surveys indicated the perceived benefits of participation usually outweighed possible hesitation. Refusal rate among those found at the households at the time of the campaign visits was only 2%. The greatest share of any incomplete coverage was attributed to absent household members. Effective community sensitization is key for successful implementation of treatment campaigns. Understanding and working through local community structures which are respected by community members, engaging traditional leaders, and working through local district-level health staff for community meetings all played a central part in achieving high levels of participation.

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SCHOOL-AGE CHILDREN ARE DISPROPORTIONATELY IMPORTANT *PLASMODIUM FALCIPARUM* TRANSMISSION RESERVOIRS IN SOUTHERN MALAWI

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Growing evidence from highly endemic malaria settings shows that *Plasmodium falciparum* infection prevalence peaks among school-age children (5-15 years old). These infections are frequently low density and asymptomatic, with less frequent antimalarial treatment-seeking than for younger children. The importance of school-age children to transmission persistence remains unknown. We evaluated the transmission contribution potential of young children, school-age children, and adults with data from southern Malawi. Six cross-sectional surveys were carried out in ~900 households from three districts at the end of the rainy and dry seasons of 2012 to 2014. To estimate the relative age-specific contributions to *P. falciparum* transmission, we populated simple mathematical models with data on population age distribution, PCR-based parasite prevalence and densities, gametocyte presence and density by qRT-PCR, and mosquito biting risk as modified by ITN use. Approximately 74.6% of gametocyte infections during the dry season and 57.9% during the rainy season are estimated to be among school-age children. While young children were more likely to have higher density infections by microscopy, infections among school-age children were the most likely to be gametocytemic, and age group was not significantly associated with density of gametocytes among gametocytemic individuals. Even if only half of gametocyte infections among school-age children and adults are assumed to be infectious, school-age children represent 68% of the infectious human population during the rainy season, and 52% during the dry season. Furthermore, in our survey, school-age children were less likely to sleep under bed nets than either adults or young children, and thus less protected from *Anopheles* feeding. When incorporating heterogeneity in ITN use, school-age children represent 82% of the gametocyte infections that are available for biting during the dry season, and 66% during the rainy season. Interventions that do not reach school-age children are unlikely to interrupt transmission in this and other highly endemic settings.

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MALARIA ELIMINATION CHALLENGES IN MESOAMERICA: EVIDENCE OF SUBMICROSCOPIC MALARIA RESERVOIRS IN GUATEMALA

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Although Guatemala remains one of the countries with higher malaria transmission in Mesoamerica, its incidence has decreased substantially since 2000. Guatemala is committed to eliminating malaria as part of the Malaria Elimination in Mesoamerica and the Hispaniola Island (EMMIE) initiative and is still in the control phase. During the past decade, the government strengthened malaria control activities, including mass distribution of long lasting insecticide impregnated bed nets, early diagnosis and prompt treatment. This study aimed to determine the prevalence of malaria in three areas of Guatemala using active case detection and molecular diagnostic tools that are more sensitive than microscopy. Cross-sectional surveys were conducted in three departments with varying transmission intensities; Escuintla, Alta Verapaz and Zacapa. Blood samples from 706 volunteers were screened for malaria using microscopy and quantitative polymerase chain reaction (qPCR) which was also used to determine the prevalence of gametocytes among infected individuals. Malaria was only diagnosed by microscopy in 2.8% (4/141) of the volunteers from Escuintla. By contrast, qPCR detected a prevalence of 7.1% (10/141) in the same volunteers, 8.4% (36/429) in Alta Verapaz, and 5.9% (8/136) in Zacapa. Overall, 7.6% (54/706) of the screened individuals were positive, with an average parasitemia level of 40.2 parasites/µl (range 1-1133 parasites/µl), and 27.8% (15/54) carried mature gametocytes. A total of 57.4% (31/54) of qPCR positive volunteers were asymptomatic and out of the 42.6% (23/54) of symptomatic individuals, only one had a positive microscopy result. A considerable number of asymptomatic *P. vivax* infections, mostly submicroscopic and with a proportion harboring mature gametocytes, was found in Guatemala. This pattern is likely contributing to the maintenance of transmission across the region. Robust surveillance systems, molecular diagnostic tests and tailored malaria detection activities in each endemic site may prove to be imperative in accelerating malaria elimination in Guatemala and possibly across all of Mesoamerica.

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ZAMBIA'S NATIONAL STRATEGY TO MOVE FROM ACCELERATED BURDEN REDUCTION TO MALARIA ELIMINATION BY 2020

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Zambia has made substantial progress addressing malaria and in many districts, malaria incidence has been reduced to levels where interruption of transmission may be feasible. This has been achieved through the commitment of national and local governments, keen interest among partners, momentum of scientific advances, and establishment of mechanisms to ensure proper coordination of activities with neighboring countries. Through a multi-stakeholder engagement process, Zambia has developed its first-ever National Malaria Elimination Strategy with the vision of eliminating malaria by 2020. All districts will be covered through a stratified step-wise approach based on a package of interventions addressing local transmission levels. The cornerstone of the strategy is the use of real-time, sensitive surveillance systems to detect, characterize and monitor all cases, at the health facility and community levels. As the first step, through a strong routine surveillance system, Zambia classified all districts according to their level of malaria transmission based on confirmed cases per 1000 population (Levels 0, 1-49, 50-199, 200-499,>500 case per 1000). The next step relies on optimizing vector control and case management—addressing current and local transmission intensities with the most appropriate and targeted population coverage of available interventions. To accelerate to elimination in selected areas, population-wide strategies to clear parasites using effective antimalarial drugs (such as mass drug administration) will be used in a time-limited manner, with the objective of bringing transmission down to a level where individual cases and small transmission foci can be appropriately managed through case investigation and community case management. Modeling suggests that this strategy will significantly reduce malaria across the country by 2020 and that regional collaboration will be key to sustaining success. Cost estimates will be presented for different scenarios, making the financial case for investing in malaria elimination in Zambia.

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TRANSITIONING AN EVIDENCE-BASED MALARIA MASS DRUG ADMINISTRATION (MDA) RESEARCH STRATEGY TO PROGRAM/ROUTINE MODE: FACTORS FOR CONSIDERATION

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The Zambia National Malaria Control Programme conducted a large-scale (60 health facility catchment areas serving nearly 330,000 participants) community randomized controlled trial to evaluate the effectiveness of treatment strategies on accelerating malaria elimination. The trial strategies included: mass drug administration (MDA), where all eligible individuals were treated with dihydroartemisinin-piperaquine (DHAp), and focal MDA (fMDA) where all eligible individuals residing in a household with at least one RDT positive member were treated with DHAp. Four treatment campaign rounds (two each year) were conducted during late dry season and early rainy season between December 2014 and February 2016. Informed by successful implementation of the trial, the new National Malaria Elimination Strategy 2016–2020 includes MDA as a key intervention. Reflecting on the successes and challenges of the recent MDA campaigns, this poster presents essential elements that implementers should consider when planning malaria MDA interventions. Trainings, logistics, and procurements were organized by health facility catchment area for decentralized operational management. Catchment teams were trained with household lists and maps for spatial orientation to maximize work flow and population coverage. Adverse event monitoring and post-marketing pharmacovigilance were conducted. Community mobilization was a prerequisite to maximize local participation. Diverse stakeholder groups were engaged for appropriate community authorization and access. Despite exclusion of children less than 3 months of age and women in early pregnancy, the trial achieved coverage rates as high as 87% and refusal rates among individuals present of less than 2% during house-to-house visits. Results from programme implementation experience will be presented in comparison to the trial methods.

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INCLUDING MOST-AT-RISK POPULATIONS IN HEALTH PROGRAM PLANNING AND IMPLEMENTATION: AN INTERCULTURAL COMMUNICATION FRAMEWORK TO SUPPORT MALARIA ELIMINATION AMONGST INDIGENOUS PEOPLES IN THE AMERICAS

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Central American countries need to improve malaria interventions with indigenous communities in order to eliminate malaria by 2020. A 2014 Amazon Malaria Initiative needs assessment found that national malaria control programs (NMCPs) in Central America had difficulty reaching these most-at-risk populations. Three cases in which malaria incidence had decreased in indigenous communities over the past 10 years were selected for further study. From 2015-2016, a literature review and in-depth, semi-structured interviews were conducted to examine intercultural approaches that may have contributed to lower malaria incidence in the selected communities. Approval to conduct interviews was granted by the Pan-American Health Organization and NMCPs of the Guatemalan, Honduran, and Panamanian ministries of health. In a Miskito community in Gracias a Dios Department, the Honduran NMCP worked with community members to achieve 100% coverage of a campaign to distribute and install long-lasting insecticidal nets. From June 2010 to June 2011, a reduction from 337 to 60 malaria cases was observed. In Bisira, in the Ngöbe-Buglé *comarca* of Panama, after the implementation of indigenous participation in health decision-making and environmental sanitation work, malaria cases fell from 71 in 2004 to zero cases by 2008. In the department of Alta Verapaz, Guatemala, local health authorities used a mobile phone system to improve surveillance, timely diagnosis, and treatment of malaria with the Q'eqchi Maya population. Findings were that the interventions studied prioritized community awareness and participation, integration of local languages, intercultural capacity development of health professionals, and adaptive design and implementation of culturally appropriate malaria interventions. The authors developed an intercultural communication framework based on best practices from the three cases. The resulting framework enables the adaptation of best practices to other contexts, in order to collaborate effectively with indigenous populations on malaria and meet the 2020 elimination target.

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ADVERSE EVENT REPORTING FROM MALARIA MASS DRUG ADMINISTRATION ROUNDS CONDUCTED IN SOUTHERN ZAMBIA

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The National Malaria Control Center of the Ministry of Health of Zambia is conducting a large-scale mass drug administration (MDA) community randomized controlled trial to evaluate the effectiveness of different MDA strategies on reducing malaria infections. The trial involved two strategies: MDA, where all eligible individuals were treated with dihydroartemisinin—piperaquine (DHAp), and focal MDA (fMDA), where all eligible individuals residing in a household with at least one RDT-positive member were treated with DHAp. Implementing MDA at such a large scale provides an opportunity to document the extent to which potential safety issues are reported or adverse events occur given the level of exposure to treatments. Field teams composed of community health workers, enumerators, and adherence monitors, and supervised by facility-based staff, received standardized training on the treatment campaign procedures, use of DHAp for eligible consenting participants, adverse event monitoring, grading of events, and emergency and event handling procedures.. Adverse events were recorded on standard forms and in line with recommendations from national pharmacovigilance network recommendations. The principle aim of this data collection activity was to document and follow up on all adverse events (AEs) and serious adverse events (SAEs) occurring during the course of implementing the MDA trial for individuals taking DHAp and reporting an adverse event to a catchment team member or local health facility. During 4 rounds of MDA community-based teams recorded all adverse events related to taking of DHAp. During the first two intervention rounds, over 280,638 participants were tested and 159,696 were treated with DHAp in 40 health catchment areas. A total of 687 AEs (0.24% of participants and 0.43% of treatments) were reported; one was recorded as a serious AE. The most common AE reported during the campaigns was stomach pains, followed by dry cough and vomiting; details and characteristics of persons with AEs will be reported. During this large MDA trial, the use of DHAp for malaria treatment was generally well tolerated.

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THE ROLE OF THE PRIVATE SECTOR IN SURVEILLANCE FOR MALARIA ELIMINATION IN HISPANIOLA: A CASE STUDY

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Hispaniola, an island in the Caribbean home to Haiti and the Dominican Republic (DR), is targeting malaria elimination by 2020. If accomplished, this binational goal would create a malaria-free zone across the Caribbean. Haiti bears the majority of the malaria burden with >95% of the total malaria cases on the island. A key priority for achieving elimination will be ensuring that all malaria cases are diagnosed and reported in a timely fashion. However, it is thought that many individuals with fever first seek care in both the formal and informal private health sectors, and there is limited information on how best to engage the private sector in Hispaniola in effective malaria case management and reporting. This project aimed to gain a better understanding of the private sector to inform both governments and partners on how to effectively integrate the private sector in malaria case reporting systems, and align private sector activities with national policies. A mixed-methods research design was used, comprised of a literature review, focus groups, and semi-structured interviews with key informants, private providers, and patients seeking care for fever. Private health sector is diverse and includes formal private, non-governmental, and mission hospitals and clinics, and informal shops, street vendors, and traditional healers. Preliminary results suggest that while the informal private sector is more utilized for care in Haiti than in the DR, in neither country does this sector have sufficient access to rapid diagnostic tests to confirm malaria cases, and to the national treatment strategy. In Haiti, care-seeking behavior is strongly influenced by spirituality, and those with more severe symptoms will often visit a traditional healer before they go to the formal sector. In the DR, visiting a traditional healer for a fever is not common, unless the cause is unable to be determined by a doctor. Traditional healers in both countries report referring patients with fever to formal healthcare facilities and would like to be more formally linked.

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COMPARING DATA FROM A MALARIA ROUTINE SURVEILLANCE SYSTEM TO HEALTH FACILITY SOURCE RECORDS IN ETHIOPIA AND SENEGAL

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The Governments of Ethiopia and Senegal are working toward achieving malaria-free zones in specifically targeted regions. Accurate, routinely reported information on malaria case rates is required to direct appropriate strategies to achieve this goal and to monitor progress. We assessed the accuracy of a rapid reporting system, with weekly malaria case rates reported to DHIS 2, in 2013 and 2014 at 20 health posts in Amhara Region, Ethiopia and at 13 health posts in Kanel, Linguère, and Ranérou districts in northern Senegal. Data on RDT-confirmed and treated malaria cases were extracted from paper registers at the health posts, including date of diagnosis, malaria RDT result, age, and area of residence Data from source records were compared to data reported in DHIS 2 during the 2013 and 2014 transmission seasons. In Ethiopia according to source records, the average weekly incidence of malaria per 1000 population across all 20 health posts was 0.85 (95% CI, 0.29-1.41) and 0.61 (95% CI, 0.02-1.21) during the major transmission seasons (September-November) in 2013 and 2014, respectively. The mean difference between DHIS 2 and source records in the number of malaria cases reported per week across all health posts was 1.33 (95% CI, 0.79-1.87) in 2013 and 0.17 (95% CI, -0.52-1.82) in 2014. In Senegal according to source records, the average weekly incidence of malaria per 1000 population across all 13 health posts was 53.08 (95% CI, 30.4-75.76) and 23.58 (95% CI, 12.94-34.22) during the major transmission seasons (July-January) in 2013 and 2014, respectively. The mean difference between DHIS 2 and source records in the number of malaria cases reported per week across all health posts was 1.86 (95% CI, 0.42-3.29) in 2013 and 0.22 (95% CI, -1.1-0.66) in 2014. Malaria cases by age (<5 and ≥5 years) and species in DHIS 2 had varying degrees of accuracy to source records. Travel history could not be ascertained from source records for most cases. Routine monitoring of the discordance between DHIS 2 data and source records combined with targeted retraining in health posts with higher levels of discordance may result in substantial improvement in the accuracy of DHIS 2 data.

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PRELIMINARY RESULTS OF THE THIRD MALARIA INDICATOR SURVEY IN ETHIOPIAN (MIS-2015)

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Malaria is among the major health problems in Ethiopia. Two Malaria Indicator Surveys (MISs) were conducted in 2007 and 2011 to measure the coverage and utilization of key malaria interventions, malaria parasitemia, and anemia. These surveys assessed the progress on scale-up of malaria prevention and control interventions. A follow up MIS 2015 was conducted between September and December 2015 to measure attainment of goals set in the 2011-2015 national malaria strategic plan. MIS 2015 was a population-based cross-sectional household (HH) survey. Two stage cluster probability sampling was used to select 555 enumeration areas from all malarious areas of Ethiopia. The survey followed standardized MIS guidelines that included the household and women's questionnaires that were uploaded on to smart phones using the Open Data Kit platform with GPS capability. A total of 100,159 HHs were mapped and 13,875 HHs were randomly selected. Overall, 64% of HHs had at least one long-lasting insecticidal net (LLIN) with an average of 1.8 LLIN per household; 32% of HH achieved universal coverage (1 LLIN per 2 persons). IRS had been conducted in 29% of HHs in the 12 months preceding the survey and 71% of HHs in malarious areas were protected by either a LLIN or IRS. Of children less than five years of age (U5), 45% slept under a LLIN the night before the survey, and 70% slept under a LLIN if the HH owned at least one LLIN. These figures were 43% and 71%, respectively, for pregnant women. Sixteen percent of children U5 had history of fever in the two weeks preceding the survey; of these, 38% sought medical attention within 24 hours of fever onset and 89% took an antimalarial drug. Malaria parasite prevalence in areas <2,000m was 0.6% by microscopy blood-slide examination and 1.4 % by rapid diagnostic test with regional variation. The results of the current survey document the sustained gains in malaria control in Ethiopia while highlighting gaps in current utilization of interventions.

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A LONGITUDINAL COHORT TO MONITOR MALARIA INFECTION INCIDENCE IN THE CONTEXT OF A COMMUNITY RANDOMIZED TRIAL OF MASS DRUG ADMINISTRATION IN SOUTHERN PROVINCE, ZAMBIA

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The Zambian National Malaria Control Center (NMCC) has embarked upon an elimination strategy in Southern Province and has recently conducted a community randomized trial to compare the effectiveness of mass

drug administration (MDA) and focal mass drug administration (fMDA; treating all persons in households with any rapid diagnostic test (RDT) positives) using dihydroartemisinin-piperaquine (DHAp) to standard of care interventions. In this setting we enrolled individuals into a prospective cohort in December 2014 during the first mass treatment round to assess community infection incidence over a 12 month follow-up period and evaluate the impact of MDA and fMDA. After clearing all identified malaria infections, the cohort consisted of monthly follow-up on 2,250 individuals from 60 health facility catchment areas and collection of parasite infection samples using RDTs and dried blood spots for molecular testing, fever and travel history, intervention coverage, and other risk factor data. Monthly entomological data were collected at a sample of cohort households, and monthly climate and environmental data linked to each cohort-month. A total of 1,388 individuals under 20 years of age and 750 20 and older were successfully enrolled. Cumulative infection incidence by RDT was highest for children under 5 (0.060 infections per person-month), and lowest for individuals 20 years and older (0.028 infections per person-month). Infection prevalence was highest in December 2014 preceding the start of the trial (7.2%) and lowest in October 2015 following the third MDA and fMDA round (1.4%). Cumulative infection incidence following trial implementation was lowest in the MDA arm (0.031 infections per person-month), highest in the control arm (0.048 infections per person-month), and intermediate in the fMDA arm (0.037 infections per person-month). Infection incidence in Southern Province has been reduced to the point where case-based elimination surveillance strategies are warranted: health facilities can now move to community case management and case and foci investigations and response to sustain these gains and seek local elimination.

1556

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SURVEILLANCE SYSTEMS FOR ELIMINATION: LESSONS FROM RAPID REPORTING ACROSS FOUR COUNTRIES

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Data-driven decision-making in national malaria control programs in Africa is critical for the efficient use of resources across countries with diverse malaria burden. Despite the need for improved information, routine malaria surveillance throughout sub-Saharan Africa is known to have many challenges including under-representation of the true burden of malaria circulating in communities, as well as lacking quality and timely data reporting. As malaria control programs pursue malaria elimination, timely, reliable data becomes crucial to respond to potential resurgence and to target malaria transmission foci with appropriate interventions. During the past five years our project team worked with national malaria control programs in Ethiopia, Kenya, Senegal and Zambia to support different forms of electronic, paper-based and blended malaria surveillance systems. In Ethiopia the system covered 213 health facility catchment areas (HFCAs) in eight woredas; In Kenya the system covered 25 HFCAs in one sub-county; in Senegal the system covered 212 HFCAs in 3 regions; and in Zambia the system covered 446 HFCAs in three provinces. We assessed different attributes and characteristics of deploying each of the systems, looking at the barriers and facilitators to improving system functionality (quality and timeliness), the performance and ultimately the use of data by national and subnational decision makers in each country over a two year period. We explored different drivers of system deployment including organizational culture, technical infrastructure, data collection, storage and reporting processes and existing talent and skills of users in each country context. Our findings suggest that the selection of open source platforms such as DHIS2 and ODK and the training and mentoring of local staff at all levels can lead to a well-supported system that can produce quality information for decision making; we will further delineate the critical system components in the presentation.

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DEVELOPMENT OF A CLOUD-BASED DISEASE SURVEILLANCE AND RISK MAPPING (DISARM) PLATFORM FOR MALARIA ELIMINATION SETTINGS - CHALLENGES FROM AN IT PERSPECTIVE

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In 2015, there were around 214 million cases of malaria and 438 000 deaths. Malaria control efforts have cut the number of deaths in half. Despite the enormous burden of on sub-Saharan Africa, 90% of all malaria deaths in 2015, a number of countries on the continent are pursuing malaria elimination efforts.1 The ability to track and target malaria transmission is vital to the success of malaria elimination programs, especially in elimination settings, where transmission is rare and clustered.2 The DiSARM platform pulls surveillance and intervention data in real-time, combines it with climate and environmental data, automates analyses by running spatial models in Google Earth Engine, and produces risk & decision-support maps. A key feature of the platform is that non-experts can run spatial models to produce risk maps. After piloting DiSARM in Swaziland and Zimbabwe, the goal is to introduce the platform to all Elimination Eight countries in southern Africa. The development

of DiSARM involves a number of steps, which have already taken place or are scheduled for this year, an in-depth evaluation of current malaria surveillance data processing, in-country implementation in pilot countries and development of platform based on pilot findings. A major barrier to implementing DiSARM is that malaria surveillance systems are set up for reporting and monitoring & evaluation. Data are often transferred to the surveillance system in aggregate and transfer intervals are long and often manual. Both proprietary surveillance systems and widely used surveillance platforms like District Health Information Software are common. A process for implementing DiSARM in both scenarios has to be developed. The group of potential end-users for DiSARM is large and with diverse needs. The interface has to be customizable to the different tasks by the different users. The potential of DiSARM in supporting malaria elimination efforts is huge. Pilots will give valuable insight for the roll-out in more countries.

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DETECTING HIGH TRANSMISSION FOCI OF MALARIA IN A LOW TRANSMISSION SETTING: RESULTS OF A PILOT MALARIA MAPPING SYSTEM IN HAITI

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The National Malaria Control Program (NMCP) in Haiti is committed to eliminating malaria by 2020. National malaria parasite prevalence is <1%, but areas of relatively high transmission (foci) are heterogeneously distributed across the country. To detect malaria foci across space and time in this low endemic setting, the NMCP established a pilot system to identify laboratory-confirmed RDT and smear positive malaria cases from health facilities in Grande-Anse, Sud, and Sud-Est Departments and to geo-locate their residences. From October 2014 to September 2015, 1,419 (58%) of 2,462 confirmed cases from 57 health facilities were identified, mapped, and a surveillance questionnaire was administered. Less than 1% of all geo-located cases traveled outside their communes in the month before seeking health care. Mean age of malaria cases was 22 years (range: 0-97) and mean number of days with symptoms before care seeking was 2.9 days (range: 0-32). Case distribution was compared to a completely random spatial distribution using the Global Moran's I test; clustering was present in two of ten analysis areas ($p<0.01$). Two space-time permutation models were created in SatScan 9.4 to search for smaller significant transmission foci with parameters of a maximum cluster radius of 3km and two minimum time spans of 14 days and 1 month. In total, 13 statistically significant ($p<0.05$) space-time case clusters were detected; average radii was 844 meters (118 cases) for the 14 day clusters and 1.14 km (170 cases) for the 1 month minimum clusters. Cases were aggregated and joined to the 2014 LandScan dataset and malaria incidence rates were calculated for 1km2 areas. Local incidence rates were compared to a global rate using the Getis Ord-Gi* analysis and to regional rates with the Anselin's Local Moran's I analysis. The global method identified 43 1km2 foci ($p<0.05$) and the regional method detected 69 foci with five outlier foci (isolated areas with unusually high incidence rates). This system detected foci at differing time and spatial scales contributing to targeting of malaria control and elimination activities.

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EPIDEMIOLOGICAL AND OPERATIONAL LESSONS LEARNED FROM A MALARIA ELIMINATION CAMPAIGN IN ZAMBIA'S LAKE KARIBA REGION

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The Zambia National Malaria Control Centre is conducting an elimination campaign in the Lake Kariba region in Southern Province, with widespread scale-up of drug campaigns, via mass screen and treat (MSAT) and mass drug administration (MDA), and vector control. The survey data collected during the course of the campaign are highly informative with regards to the many factors that affect disease transmission; for example, analysis of local prevalence in the context of insecticide treated nets (ITNs) and indoor residual spraying (IRS) allowed the quantification of both individual and community-level protection for each method of vector control. Not to be disregarded, however, is the added value of the data for understanding the challenges of implementing surveillance and interventions across a large, dynamic population, spread out over a substantial and geographically varied region. Many of the features discovered in the data, such as a strong demographic dependence of coverage rates, and seasonal patterns of migration between smaller villages and larger centers, have major implications for the feasibility of elimination under existing protocols and suggest operational checks useful for similar programs across Sub-Saharan Africa.

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EMERGING INCURABLE MALARIA IN SOUTHEAST ASIA - A CALL FOR TARGETED, DECISIVE ACTION IN THE REGION

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Clinically nearly incurable strains of malaria have now reached Vietnam from Cambodia. Historically drug-resistant strains emerge in Cambodia and then spread to Africa. The new strains are not just artemisinin-resistant; they are resistant to nearly all antimalarial drugs. From 2001 to 2015, approximately 6.2 million malaria deaths were averted through massive global investments. Malaria mortality dwarfs that of highly publicized recent outbreaks (11,325 for Ebola, 774 for SARS and very few for Zika virus). The impressive gains with malaria are at risk of being reversed; decisive action must be taken to eliminate them. Published reports demonstrate dihydroartemisinin-piperaquine (DHA-PIP)-resistant malaria was present in Cambodia in 2010. Unfortunately, attempts to contain these parasites have failed - new approaches are urgently needed. In Binh Phuoc Province, Vietnam, in 2015, a PCR-corrected 32% (14/44) late clinical treatment failure rate was documented (K13 genotyping, drug levels pending). A confirmatory trial with early similar results is on-going. Current results and available data from Cambodia will also be presented. To address this emerging threat regionally, current M&E reports, strategies and recommendations for malaria elimination and epidemic preparedness are being critically reviewed and compared. Our team has also developed a simple red light-green light system for cross-border emergency operations centers to be able to visualize, target and monitor real-time rapid responses and on-going adherence. The same solutions and methods needed to eliminate malaria will enhance the infrastructure and

provide indispensable experience in preparation for the next global threat. We will discuss why we believe these new malaria strains should be part of the Global Health Security Agenda. In conclusion, DHA-PIP treatment failures are now in Vietnam; the first reported spread beyond Cambodia. We recommend emerging incurable malaria be addressed and resourced as a crisis. Malaria and any threat agent can be rapidly addressed using the same new approaches.

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MALARIA ERADICATION: ARE REGIONAL INITIATIVES CRITICAL TO ITS SUCCESS

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There has been major progress towards global malaria elimination within the past decades, with an estimated 1.2 billion cases and 6.2 million deaths averted globally since 2001. However, there is increasing evidence that individual ministries of health and national malaria programs may face major challenges in 'getting to zero' in isolation. As such regional cooperation is critical for continued progress; however there is limited guidance for the structure, planning or functional roles of regional cooperation. To address this, we review a range of global malaria initiatives, and provide practical guidance for future programs.

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VIETNAM MALARIA STATUS UPDATE AND PLAN TO ELIMINATE EMERGING INCURABLE MALARIA STRAINS

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Clinically artemisinin combination therapy (ACT)-resistant of *Plasmodium falciparum* (Pf) has now emerged in Vietnam. New WHO guidelines recommend their elimination by 2020. Since 1991, Vietnam has successfully reduced malaria cases by >97%. So that these impressive gains are not reversed, elimination of the emerging resistant strains is imperative. In 2015, Pf decrease by 47% nationwide as a result of many factors. The major exception was Binh Phuoc Province, where clinical ACT-resistance emerged, which likely caused the 32% increase in incidence (the same pattern was seen in Cambodia). A country plan to eliminate ACT-resistant Pf has been developed. The major challenges identified are as follows: 1) drug resistance, 2) forest-goers/seasonal workers, 3) financial support, 4) access to timely, essential information, 5) cross-border and intersector collaboration. Potential solutions to the challenges will be presented. Some examples are as follows. Interagency meetings are being conducted to raise domestic funding. The US Navy sponsored "enhanced surveillance and operations research" has identified unmet needs which can guide new intervention programs. Lastly, partnerships with the military are being explored to utilize the existing network of health care facilities and military personnel operating in and near malaria endemic zones. In conclusion, we present a path to resource ACT-resistant Pf for rapid elimination. New tools and collaborations now make elimination possible. As malaria disappears, the same enabling factors can be applied to other health challenges related to poverty (e.g. malnutrition). We can both lead the elimination of ACT-resistant malaria and the next global health security issue that emerges.

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U.S. NAVY-NIMPE COLLABORATIONS AND OPERATIONS RESEARCH SUPPORT OF THE MALARIA ELIMINATION PROGRAM IN VIETNAM

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US Navy collaborations with the Vietnamese National Institute of Malariology, Parasitology, and Entomology began in 2014 with an Enhanced Surveillance and Operations Research for Malaria Elimination project. Phu Yen Province in central Vietnam, near areas of highly drug-resistant malaria, is the lead study site. To better understand how to accelerate malaria elimination, our team conducted surveys in preparation for future operations research. In 2015 a survey was completed of 100 households with and 100 households without confirmed malaria in three communes of Western Phu Yen. The survey included data collection on known risk factors, occupation, use of bed nets and preferences for potential interventions. The malaria burden by self-reported occupation was: paper planation work (47%), agarwood harvesting (15%), farming (13%), charcoal production (9%), trapping (6%), timber harvesting (4%) and hunting (3%). Although total cases were lower, greater proportions of farmers, charcoal producers and hunters suffered from the disease suggesting they may be an important part of the transmission reservoir. Overall, treated net use was low (19% in risk areas), despite households having treated nets (mean = 2.8). Households reporting not using a treated net had a higher risk of malaria (OR 2.6, p=0.05). A majority of forest-goers (85%) reported dislike of nets provided by public health programs, e.g. Global Fund long lasting insecticide-treated nets; 82% of forest-goers indicated a desire for hammocks with a zip-in treated net. When asked about preferences for future interventions, 94% were willing to use malaria prophylaxis and 90% mosquito repellent. In conclusion, the majority of individuals at greatest risk of malaria in our study area did not report routine use treated bednets or other malaria prevention products. Based on these preliminary findings an updated survey is planned for 4800 households and all transmission hotspots in 2016 to confirm the 2015 findings. These activities will provide an evidence base to plan operations research to tailor interventions for malaria elimination.

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AN INNOVATIVE INFORMATION SYSTEM TO ELIMINATE EMERGING INCURABLE MALARIA

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Malaria resistant to nearly all drugs is again emerging in Southeast Asia, which must be rapidly eliminated. The goal of surveillance for malaria elimination is to capture every malaria case and execute a prompt and effective response. Here we describe the real world evaluation of an effective information system for rapid reporting, case investigation and response. Three communes in western Phu Yen (PY) Province, central Vietnam were selected as the lead study area. Sony smart phones, KLL collect data, and the Ona server were down-selected for data capture and management. MapInfo Professional® was utilized for enhanced mapping.

Five data capture forms were developed and iteratively improved during the study period of January to September 2015. GPS coordinates in the transmission sites were captured for 89% of 64 cases from January to September 2015. Fourteen transmission foci were identified, defined as more than one case within a one km radius, which accounted for 80% of cases. The majority of cases (97%) were from people living in PY. Forest (86%) and forest fringe (14%) were identified as the probable transmission sites. Only 16% of cases reported sleeping under a treated net, 30% an untreated net and 54% without any net. The sleeping sites were classified as houses (10%), huts (60%) and hammock only (25%). Routine follow-up forms were tested in 20 households; 45% were probably using, 35% were possibly using and 10% were not using the net provided to their household. By self-reporting, 10% reported not regularly using their net, which increased to 25% when the picture evidence of non-use was included. Weekly (zero) and monthly quality reporting forms were also successfully evaluated. New results and plans to expand to high-priority areas also will be presented. In conclusion, the system was found to be user-friendly. The use of pictures revealed discordance between self-reporting and actual net use. The described transformative technology will help National Malaria Control Programs and partners improve the quality and targeting of interventions. This new approach will facilitate rapid elimination of malaria.

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THE FORMULA "AVERAGE PLUS TWO STANDARD DEVIATIONS" APPLIED TO THE FOLLOW-UP OF THE MALARIA EPIDEMIC WARNING THRESHOLD OF THROUGH 24 SITES SENTINELS OF SURVEILLANCE IN SENEGAL

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To predict and manage malaria epidemics, the Senegal National Malaria Control Program (NMCP) created an epidemiological surveillance system of rapid detection. This malaria sentinel system, set up in 2008, includes 24 sentinel sites, 18 of which are distributed in low to moderate transmission zones at risk for epidemics. Using a standard Excel spreadsheet, sites report the total patients; suspected malaria cases, patients tested, and confirmed malaria cases every week. The quality of the transmitted data is assured through quarterly supervision of sites integrating on-site data verification. Since 2009, 100% of suspected malaria cases have been tested by rapid diagnostic tests, and 100 % of positive cases received artemisinin-based combination therapy. In 2015, the NMCP introduced a method of calculating epidemic threshold using historical data for each site dating back five years. For every site, a standardized Excel worksheet is used to calculate and draw a curve of the epidemic threshold. The following formula is used for calculation of the epidemic threshold: weekly average of the cases for a given epidemiologic week over the previous five years plus two standard deviations. During the 45 epidemiologic weeks of 2016, the reported cases were systematically compared with the epidemic threshold curve weekly. This formula proved to be very sensitive, with detection of 99% of potential epidemic situations during 2016, compared to 25% using a threshold based on a formula of simple averages. In every sentinel site if the threshold is met or exceeded, a systematic documentation of the reported cases is made. In zones of moderate or high transmission, specific actions are taken based on the results of this documentation. In zones of low transmission, the threshold is one case, and every case is systematically documented and investigated. Since implementation, four sites reached or surpassed the warning level more than four times. Investigations showed that 80% of cases were imported, and 20% of autochthonous cases did not sleep under insecticide-treated nets.

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INCREASE OF MALARIA TRANSMISSION IN MILITARY CAMPS IN TANZANIA

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Malaria remains a public health problem for many Tanzanians. Parasitemia prevalence in the population has declined markedly from 78.4% (2003) to 13.0% (2008). Recent studies have reported high prevalence in Kigoma (26%), Mara(25%) and Kagera(37%). We investigated malaria burden in eight military camps in seven regions of Tanzania, Coastal, Tanga, Kigoma, Morogoro, Mara, Tabora and Kagera regions. Malaria surveillance was conducted using the Deki mRDT Reader for 2 years and malaria prevalence survey of asymptomatic military recruits at four military camps. Finger pricks were used to conduct malaria mRDT and blood smear microscopy. Studies to determine the malaria attack rate for recruits from endemic regions and nonendemic areas were performed. Consented recruits were screened negative enrolled and followed-up biweekly for six months. Malaria case detection with the Deki mRDT Reader showed increased positivity rates (PRs) over time. At Ruvu, PRs increased from 11.8 % (2013) to 15.7% (2014) to 30.9% (2015). PRs increased from 25.2%(2013) to 37.5%(2014) and to 46.2%(2015) for Mgambo and from 36.4%(2014) to 37.0%(2015) for Rwamkoma Bulombora site showed a decreasing PRs, other military camp sites showed an increasing PRs. malaria attack rates in the Ruvu and Mgambo camps were 13% and 43.3% respectively. Malaria prevalence rates by mRDT for asymptomatic recruits were 2.8% (Bulombora), 5% (Ruvu), 47.5% (Mgambo) and 39.4% (Rwamkoma). Malaria prevalence rates by microscopy were 3.4%, 5.2%, 49% and 38.5% for Ruvu, Mgambo and Rwamkoma respectively. Generally, there is an increasing trend of malaria prevalence in these areas which could be an indicator of similar trends countrywide. Further studies and an intervention plan are clearly recommended to address this public health problem.

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RISK FACTORS INVOLVED IN THE EPIDEMIOLOGY OF MALARIA IN MILITARY CAMPS IN TANZANIA

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A total of 1380 patients were interviewed from Rwamkoma and Maramba camps. More than one quarter (29%; 400/1380) of respondents had never used insecticide treated nets (ITNs) as 31.8% did not slept always under an ITN 30 days prior to interview. Respondents who reported not using ITNs had a statistically significant probability to be diagnosed with laboratory confirmed malaria ($p<0.0001$). Factors such as male gender (35.7%; 280/784) or age <17 years (49.4%; 44/89) were found to be statistically significant contributing factors to developing malaria. Although the results were not statistically significant, patients coming from malaria non-endemic districts of origin contributed to higher rate(35.2%; 25/71) of laboratory confirmed cases than patients from malaria endemic districts of origin (31.2%; 380/1218). Patients diagnosed clinically to have malaria were statistically more likely to have laboratory confirmed malaria than those diagnosed clinically free from malaria ($p<0.0001$). Patients that presented at health facility with fever of greater than 3 days were statistically more likely to have confirmed malaria than patients presenting with a fever of less than 3 days ($p<0.0001$). Although statistically not significant, patients with clinical symptoms > 5 of malaria (≥5) were more likely to have confirmed malaria than those with <5 symptoms of malaria (35.5%; 39/110). Patients who reported to have not completed a full

regimen of antimalarial treatment in the two months prior to the study were more likely to test positive for malaria than patients who completed a full course of treatment (60%; 3/5). In summary, factors associated with the epidemiology of malaria included prior experience using ITNs, gender, age, delay in seeking treatment and clinical symptom presentation.

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ESTIMATING THE MALARIA ATTACK RATE IN TANZANIAN MILITARY CAMPS. MALARIA EPIDEMIOLOGY IN SELECTED MILITARY CAMPS IN TANZANIA

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In Tanzania, malaria ranks number one cause of morbidity and mortality, accounts for over 32% of the National disease burden. There is high heterogeneity of malaria transmission depending on topographical features and climatic conditions. The aim of this study was to look at malaria attack rate among recruits entering training camps in highly endemic areas. Recruits eligible to study in Mgambo camp -Tanga, were randomly selected by multistage sampling; consented and followed for six months. Fortnightly malaria smear was collected. Blood samples for serological tests were collected. Microscopy was the gold standard method for malaria diagnosis. Data was subjected to univariate and multivariate analysis, logistic regression model was used to identify the risk factors. Among 549 recruits who were involved in this study, 31.7% (174) were malaria positive. Among positive cases, those who didn't sleep under treated net were found to have significantly increased odds of being malaria positive [OR: 7.71; 95% CI: 1.01-58.61; P=0.048]. Travelling outside the camps increased odds of being malaria positive [OR: 1.25; 95% CI: 0.87-1.79; P=0.232]. There was significant difference between malaria positivity and place of travel [X^2=40.1; P=0.015]. Female recruits had 54% significantly reduced chances of being malaria positive [OR: 0.46; 95% CI: 0.28 - 0.77; P=0.003]. This study revealed failure to use bed nets and travel are major drivers of malaria infection. Identification of gaps in net use, knowledge, relevant types of human movement and development of strategies addressing travel is highly recommended. Mgambo camp in high malarial endemic area is an ideal site for malaria drug prophylaxis or vaccine studies, where TPDF recruits from various transmission intensity areas trained under similar environment.

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CHALLENGES OF MEASURING ITN EFFICACY IN HIGH TRANSMISSION SETTINGS: AGE, SEASON AND DETECTION METHODS

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After universal distribution of insecticide treated nets (ITNs), malaria prevalence remains high in Malawi. Understanding reasons for the limited impact of ITNs on malaria control is critical for identifying methods to decrease the malaria burden. Previous studies focus on the impact of ITN use among children under 5. However, prevalence of infection is highest in school aged children (SAC), ages 5 to 15. We examined the effect of age, season, and detection method on estimates of ITN impact in Malawi. Six cross-sectional surveys using cluster random sampling were conducted in the rainy and dry seasons in southern Malawi from 2012 to 2014. Data were collected on household ITN usage and demographic variables. Blood samples for detection of *P. falciparum* infection were obtained from all household members who were present and over six months of age. Statistical analyses used generalized linear mixed models to account for clustering at the household and neighborhood level. We conducted microscopy and qPCR on ~17,500 individuals. Prevalence was higher in SAC compared to children under 5 and adults using both detection methods (15% vs. 8% and 5% by microscopy and 23% vs. 11% and 11% by qPCR respectively). The association between ITN use and infection was modified by season in SAC, but not in others. Using microscopy, ITN use was associated with protection in all age groups and all seasons (SAC in the rainy season: OR = 0.67 (95%CI: 0.46, 0.97), SAC in the dry season: OR = 0.57 (0.37, 0.88), and non-SAC: OR = 0.73 (0.56, 0.94)), but there was no protective association among SAC in the rainy season when using qPCR results (OR = 0.78 (0.56, 1.10)). Sensitive detection methods reveal a lack of uniformity in the impact of ITN use, with seasonal variation among SAC when using qPCR results. This may be due to inadequate protection of ITNs when mosquito density is high or the persistence of sub-microscopic infections over time. Single time-point cross-sectional surveillance of children under five using microscopy alone may fail to capture the community impact of ITN use on prevalence. SAC may represent persistent reservoirs of transmission and may require targeted interventions.

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DECLINING MALARIA BURDEN IN UGANDA BETWEEN 2009 AND 2014: EVIDENCE FROM THE MALARIA INDICATOR SURVEYS

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Malaria remains a major public health problem in Uganda. Although there is still on-going transmission and the entire population remains at risk of malaria infection, results from the 2014 national malaria indicator survey showed improvement in key malaria indicators. In this study, we present evidence of declining malaria burden in Uganda by comparison of key population-based malaria indicators from the 2009 and 2014 national malaria indicator surveys. We extracted and compared data on malaria biomarkers between the 2009 and 2014 national malaria indicator surveys for population-based estimates of parasitemia and anaemia as key indicators. Both surveys used a comparable sample of children aged 0-59 months who were all tested for malaria and anemia as the major outcomes. Improvement in key indicators was determined by analyzing the differences in proportions of parasitemia and anemia between the 2009 and 2014 surveys. There was a reduction in parasitemia from 42% in 2009 to 19% in 2014 (difference= 23% CI: 21.1-24.9, p < 0.001). Severe anemia (hemoglobin <8 g/dl) decreased from 9.7% in 2009 to 4.6% in 2014 (difference=5.1% CI: 3.9-6.1, p <0.001). In both surveys, parasitemia was significantly higher in older children 48-59 months 25.9%, p<0.001 and 53.2%, p<0.01 in 2009 and 2014 respectively. Although *Plasmodium falciparum* mono-infection and *P. falciparum* combined with other species constituted 97.1% of the malaria speciated in the 2014 survey (down from 99.1% in 2009), there was general increase in prevalence of non-falciparum species: *P. malariae* from 2% to 6%, *P. ovale* from 0.02% to 1.3%, *P. vivax* from 2% to less than 1% in 2009 and 2014 respectively. These results provide strong evidence of the declining malaria burden in Uganda between 2009 and 2014.

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PROXIMITY TO ENVIRONMENTAL RISK FACTORS INFLUENCES SPATIAL PATTERNING OF *PLASMODIUM* INFECTION PREVALENCE IN DANGASSA, MALI

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Malaria is highly seasonal throughout Mali, with large-scale differences in transmission across the broad range of Saharan to Sahelian to Sudano-Guinean habitats. In the tropical savanna climate of Sudano-Guinean southern Mali, transmission is seasonally intense and spatially heterogeneous. This study sought to characterize community-level spatial patterns of malaria and examine fine-scale environmental factors that may influence transmission. A cross-sectional study of 1,063 people in 190 households of Dangassa, Mali was conducted during September 2012 (end of rainy season). *Plasmodium* (primarily *P. falciparum*) infection was determined by standard microscopy, and peri-domestic land cover (crops, trees, etc.) around each dwelling was observed/GPS-located in the field (2015) and through satellite images (2013). Distances to environmental features and health-relevant locations were calculated through GIS. Household-level, multivariate linear and geographically-weighted analyses and individual-level logistic regression was performed to evaluate demographic and environmental associations with malaria, as were spatial cluster analyses. Overall, 431/1063 (40.5%) of community members were *Plasmodium*-positive, with 5-10 year-olds exhibiting the highest prevalence of infection (64.8%). Household-level prevalence was positively associated (increased) with distances to the health center and to paths, but negatively associated (decreased) with distance to forest and breeding sites. Curiously, nearby crop cover reduced household and individual infection risk. Household-level clustering of infection was demonstrated, with geographically-weighted regression producing a better model fit than linear regression. Environmental risk factors contribute to variance in malaria risk at fine spatial resolutions. Geographically-weighted regression may be useful in determining areas of increased malaria prevalence.

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EXPLORING ENVIRONMENTAL FACTORS MEDIATING SPATIOTEMPORAL VARIATION IN VECTOR CONTROL IMPACT IN SUB-SAHARAN AFRICA 2000-2015

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Previous work by the Malaria Atlas Project (MAP) has led to a detailed spatiotemporal reconstruction of the changing landscape of *Plasmodium falciparum* risk in sub-Saharan Africa since the year 2000, and an understanding of the overall contributions of vector control (primarily Insecticide treated bednets, ITNs, and indoor residual spraying, IRS) in driving these changes. However, the patterns of declining transmission are not uniform, and the likely impact of existing vector control varies substantially from place to place. Understanding what influences these variations in impact can help inform thinking around novel vector control approaches that may be needed to address this 'residual' transmission. Here, we develop a suite of relevant environmental covariates and a geospatial modelling framework to explore factors influencing observed trends in transmission. Of particular interest are aspects of the biophysical or human environment that have either: (i) mediated the impact of existing vector control interventions, particularly insecticide treated bednets (ITNs) and indoor residual spraying (IRS); or (ii) led to declines in transmission independent of vector control or other intervention efforts.

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RISK FACTORS FOR DEATH DUE TO SEVERE MALARIA IN CHILDREN UNDER FIVE YEARS, KALEMBE-LEMBE PEDIATRIC HOSPITAL OF KINSHASA, DEMOCRATIC REPUBLIC OF CONGO, 2012-2014

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Malaria remains a major public health problem in the DRC. Its prevalence in children 6-59 months old is 31% and up to 30% of pediatric deaths are due to malaria. The aim of this study is to determine the risk factors of death in severe malaria in children under 5 years old. A case-control study is conducted in 2015 at the Kalembe-Lembe pediatric hospital (Kinshasa). Cases (n = 71) were children hospitalized for severe malaria and whose outcome was fatal. Controls (n = 142) were children hospitalized for severe malaria and whose outcome was favorable. A questionnaire was used to collect the indicators (age, sex, weight, hyperthermia, nutritional status, delay of treatment at the hospital ≥ or < 24 hours, socioeconomic level…) from children's mothers or guardians. The delay of treatment is the time between the first symptoms and the treatment at the hospital. The socioeconomic level is about 5 variables. Each variable has a value of 1 in the affirmative. The socio-economic level was good if the score was ≥ 4 and low if it was < 4. Data were analyzed using Epi-Info7 software. The risk of death was estimated through the OR 95%. Both cases and controls were separately 14 months of median age with a maximum of 59 months. The median weight was 9kg (3-22Kg) for cases and 10kg (5-24kg) for controls. Concerning cases, 32.4% of children (23/71) had a poor nutritional status (Z-score < -2 SD) and 80.3% (57/71) of their households had a low socioeconomic level. In 59.2% of cases (42/71), the delay of treatment was ≥ 24 hours. Risk factors associated with the occurrence of death to cases included poor nutritional status [adj OR = 2.24 (1.16-4.33)], low socioeconomic level [adj OR = 2.17 (1.08-4.35)] and delay of treatment ≥ 24 hours [adj OR = 2.05 (1.04-4.03)]. However, no association was found between death from severe malaria and rural residence, self-medication, hyperthermia and the knowledge of the mother/guardian on malaria. In the fight against malaria, malnutrition, delay of treatment ≥ 24 hours and the low socioeconomic level are risk factors considered as being associated with this disease. It is therefore imperative to intensify the awareness of these risk factors.

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MALARIA RESURGENCE IN WESTERN KENYA HIGHLAND

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In the past decade malaria-induced morbidity and mortality were significantly reduced through the deployment of insecticide-treated nets (ITNs), indoor residual spraying (IRS) and artemisinin combination therapy (ACT). Despite very high coverage of ITNs, here we reported rapid malaria resurgence in the highlands of western Kenya. Longitudinal cross-sectional surveys of malaria prevalence in school-aged children were conducted monthly in Kisii from 2003 to 2015. Monthly clinical malaria incidence was obtained from a sub-district hospital. Indoor-resting malaria vector densities were determined and cross-sectional household surveys of ITN ownership were carried out. Malaria infection rate in school-aged children was reduced from monthly average of 15.9% in 2002 before

the introduction of SP+AQ, to 3.0% before the introduction of ACT in early 2006. It remained very low from 2006 to 2013 (monthly average of 0.6%), but it increased sharply to 5.9% in 2014 and 10.2% in 2015. Indoor resting vector density was 1.5 females/house/night (f/h/n) during high season in 2003, it remained very low from 2004 to 2008 (0.03 f/h/n), however, it gradually increased since 2009 and reached 1.8 f/h/n in 2015. Malaria vectors were shifted to from An. gambiae to An. funestus as the dominant vector. Household ITN ownership increased gradually from 11.7% in 2003 to 87.4% in 2015. Despite consistently high coverage of ITNs, malaria infections and indoor resting vector density rebounded dramatically in the past few years in western Kenya highland. There is a renewed fear of malaria epidemic in western Kenya highland, calling for urgent and improved malaria interventions being placed in these epidemic-prone highland areas.

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RESULTS AND RECOMMENDATIONS FROM THE 2015 MALARIA INDICATORS SURVEY (MIS) IN ZAMBIA

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National, population-based cross-sectional surveys such as Demographic and Health Surveys (DHS) and Malaria Indicator Surveys (MIS) provide current and historic estimates for comparing malaria infection and intervention coverage to measure progress toward national and international targets. In Zambia, surveys were conducted in 2001-2002 (DHS), 2006 (MIS), 2007 (DHS), 2008 (MIS), 2010 (MIS), 2012 (MIS), 2013-2014 (DHS), and 2015 (MIS). We reviewed information from the 2015 MIS and its cluster randomized 3,750 households to evaluate recent progress toward elimination and compared findings with prior surveys to examine lessons from 15 years of malaria control in Zambia. In 2015, 77.0% of households had at least one insecticide-treated mosquito net (ITN) and 63.9% of households reported sufficient ITNs to cover all sleeping spaces; 55.1% of individuals reported sleeping under an ITN the night before the survey. Indoor residual spraying reportedly occurred in 28.9% of households in the previous twelve months. The overall malaria prevalence in children was 20.3% and varied by province from 0.5% to 32.5%. Despite high coverage and use of key interventions, malaria parasite prevalence increased overall to a prevalence of 20.3% in 2015 compared to 14.9% in 2012. The increase in malaria prevalence despite high sustained coverage suggests that additional strategies will be needed to move Zambia towards elimination. The 2015 MIS also collected information relevant for monitoring the progress of the roll out of integrated community case management (iCCM), intermittent preventive treatment during pregnancy (IPTp), case management, and treatment-seeking behavior. Information on these indicators will also be reported. Overall, the 2015 MIS provides the most comprehensive assessment of Zambia's current malaria state, demonstrates that tremendous progress has been made in Zambia, and is helping to inform the planning process for future elimination efforts.

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MALARIA EPIDEMIOLOGY IN LOW ENDEMICITY AREAS OF THE NORTHERN COAST OF ECUADOR

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The recent scale up in malaria control measures in Latin America has resulted in an impressive decrease in the number of reported cases in several countries including Ecuador, with a very low malaria incidence in recent years (544 reported cases in 2012 and 377 cases in 2013) and occasional outbreaks of both *Plasmodium falciparum* and *P. vivax* in the coastal and Amazonian regions. This success in malaria control in recent years has led Ecuador to transition its malaria policy from control to elimination. Nevertheless, it is unlikely that current interventions will lead to malaria elimination in the country unless asymptomatic parasite carriers are identified and treated. This study reports the general knowledge, attitude and practices (KAP) about malaria, as well as its prevalence in four communities of an endemic area in northwest Ecuador. A total of 258 interviews to assess KAP in the community were evaluated showing that most people in the study area have a basic knowledge about the disease. Six hundred and forty-eight blood samples were collected and analyzed by thick blood smear (TBS) and real-time PCR, as well as by serology using ELISA and immunofluorescence. In addition, the distribution of the infections was mapped in the communities. The total malaria prevalence by PCR was 7.5%, comparable to that reported in endemic areas of neighboring countries with higher malaria transmission, indicating a much higher prevalence than expected. Results suggest that the transition from control to elimination strategies in a country like Ecuador would demand an improvement in malaria diagnostics to detect parasite asymptomatic carriers, as well as studies on the bionomy of *Anopheles* mosquitoes with potential vectorial capacity.

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MALARIA INDICATORS IN MALANGA, KIMPESE HEALTH ZONE IN DEMOCRATIC REPUBLIC OF THE CONGO

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Background Malaria remains a public health problem in the DRC with a morbidity and mortality highest in the world. Knowledge of different indicators to better undertake the fight against this epidemic Methodology We conducted a community survey in 60 households in the villages Malanga, Nkumba, Zamba and Malanga Station. We also conducted human landing catches.Finally we conducted *Anopheles* susceptibility testing according to the WHO protocol Results The overall prevalence of infection by *Plasmodium* to Malanga was 41.4% with 43.4% for the age group of 0-5 years, 69% for the portion of 6 to 10 years and 59.1% for range 11 to 15 years. The capture of *Anopheles* returned 186 specimens with 5 species *A. gambiae* (83.8%), *A. funestus* (11.3%), *A. nili* 3.2%; *A. moucheti* 0.5% and 1.5% *A. tenebrosus*. The evaluation of the sensitivity of the *Anopheles* to insecticides found broad a total susceptibility to bendiocarb; Susceptibility to deltamethrin and permethrin was 53.4% and 23.7% respectively conclusions: Malaria Indicators to Malanga not allow to glimpse an effective control of malaria.

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UNDERSTANDING VULNERABILITY AND RESILIENCE OF INDIVIDUALS TO *PLASMODIUM FALCIPARUM* INFECTION IN A STABLE MALARIA TRANSMISSION AREA OF DANGASSA, MALI

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The goal of this study was to examine the factors associated with the absence or presence of persistent *Plasmodium falciparum* infection (parasitemia) among subjects living in the stable high transmission area of Dangassa in Mali. In a cohort of 750 children and adults examined during 4 consecutive surveys, we identified 89 subjects with positive thick smears at all visits (persistent positives) and 91 subjects with negative thick blood smears at all visits (persistent negatives) between August 2012 and June 2014. A household survey was also performed to define the level of exposure to malaria control interventions and tools and characterize sociodemographic conditions. During each survey, clinical and laboratory examinations were performed in all subjects to estimate the frequencies of symptomatic and asymptomatic parasitemia. In addition, passive case detection was performed at the health center to estimate the incidence of uncomplicated and severe malaria in the community. Most persistently-negative subjects were adults (62.6%). In contrast, most persistently-positive subjects were children less than 17 years of age (98.9%). Persistently-negative subjects were more likely to seek care for malaria at the health clinic (72.5%) than persistently-positive subjects (34%) and the use of insecticide-treated nets was greater among persistently-negative (61.5%) than persistently-positive subjects (38.2%). Finally, persistently-positive subjects had 64% less risk of severe malaria than persistently-negative subjects (Relative Risk 0.36; 95% CI = [0.16, 0.8]. In conclusion, most persistently-positive subjects were asymptomatic and sought malaria treatment at the health center clinic less frequently than persistently-negative subjects. However, the substantial numbers of persistently-positive subjects in Dangassa provide a reservoir for the continued transmission of malaria in the community, particularly during the dry season.

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MALARIA PARASITE POPULATION STRUCTURE AND HUMAN MOBILITY IN NORTHWEST THAILAND

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Southeast Asia is the epicenter of antimalarial drug resistance. Its disposition potentially relates to regional factors, including malaria parasite population structure. Differences in parasite population structure have been associated with resistance, as well as population decline following elimination efforts; human mobility is also thought to play a role. To investigate the relationship between parasite population structure and human mobility, we aim to model the genetic relatedness of parasites sampled from four sites in Northwest Thailand under models excluding and including human connectivity. We estimate genetic relatedness between sites using published data from over 1000 *Plasmodium falciparum* positive samples genotyped at 96 single nucleotide polymorphisms (SNPs). For a pair of samples from different sites, the percentage of the parasite genome that is identical by descent (IBD) is inferred under a hidden Markov model. To account for the relatively small number of SNPs, the IBD estimates are calibrated using a regression model trained on results derived from distinct samples sequenced across the entire genome. Preliminary estimates of IBD suggest the parasite populations are partially clonal, and that a subset of parasites at different sites is highly related, implying a network of interconnected populations. Discordance between the genetic relatedness of sites and their spatial distribution suggests human mobility could play a role. We thus hypothesize that the inclusion of human connectivity in the proposed model of genetic relatedness will increase its explanatory power. Model selection will provide a quantitative assessment of our hypothesis, yielding valuable insight into the host-parasite relationship at the population level, which can be leveraged to improve the design and maintenance of critical malaria elimination strategies.

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IMPACT OF ALL-CAUSE ANEMIA ON THE RISK OF FALCIPARUM MALARIA IN MALIAN CHILDREN

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Anemia and falciparum malaria co-exist throughout much of sub-Saharan Africa. Recurrent malaria can cause anemia, and some forms of anemia, such as iron-deficiency, can reduce the risk of severe malaria. The relationship between all-cause anemia and uncomplicated malaria is less well understood. We investigated the relationship between hemoglobin concentrations and subsequent risk of malaria in a large cohort of children in Southern Mali. The KIDS-Malaria cohort comprised over 1500 children who were followed over 4 successive malaria transmission seasons. Hemoglobin levels were measured at the start of 2 transmission seasons, and with each malaria episode at diagnosis and during day 4 of treatment. We estimated the risk of subsequent episodes of malaria using inverse probability weighting in models adjusted for age, ethnicity, and alpha-thalassemia. Overall, we recorded over 4000 episodes of malaria during over 2500 child years of follow-up. Mean hemoglobin values were 11.0 g/dL in the two baseline surveys, 10.6 at the time of malaria diagnosis, and 9.5 on day 4 of treatment during these episodes. Using hemoglobin values measured either at baseline surveys or at day 4 during their previous malaria episode, we categorized malaria episodes as occurring in a child with pre-existing severe anemia (<8 g/dL, n=646), mild anemia (8-11 g/dL, n=2379), or normal (>11 g/dL, n=1179). Compared to episodes occurring in children with normal hemoglobin levels, the risk of malaria was reduced in those with severe anemia (relative risk [RR] 0.74; 95% confidence interval [CI] 0.604-0.906) and unchanged in those with mild anemia (RR 0.939; 95% CI 0.845-1.044). This effect was most pronounced in the final year of observation, when, relative to episodes occurring in children with normal hemoglobin levels, the risk of malaria was reduced in those with both severe (RR 0.255, 95% CI 0.16-0.4) and mild anemia (RR 0.60, 95% CI 0.5-0.73). These data suggest that all-cause anemia reduces the risk of uncomplicated malaria in this cohort of Malian children.

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CROSS-BORDER MALARIA: THE CONTRIBUTION OF POPULATION MOVEMENT TO SUSTAINED MALARIA TRANSMISSION IN MUTASA DISTRICT, ZIMBABWE

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Malaria persists as a public health problem in Zimbabwe despite continued vector control efforts with long-lasting insecticide treated nets and indoor residual spraying. Population movement between Mozambique and Zimbabwe may play a role in sustaining malaria transmission in Mutasa District, Zimbabwe. The aim of this study is to assess cross-border malaria transmission between Mozambique and Zimbabwe. Between 2012 and 2016, passive case detection of malaria cases, determined by rapid diagnostic test (RDT), in 43 clinics in Mutasa District were collected using the country's established health management information system. In 2015, six clinics in Mutasa District began reporting weekly data on confirmed malaria in patients from Mozambique seeking care in Zimbabwe. Preliminary data show that approximately 18.2% of all confirmed malaria cases in border clinics are patients residing in Mozambique. This study will address three hypotheses as to why higher numbers of incident cases of malaria are reported on the border of Mozambique. First, these areas may have a higher incidence of malaria because of ecological factors favoring vector breeding sites. Second, higher case numbers at clinics near the border may reflect the health seeking behaviors of symptomatic individuals residing in Mozambique. Third, movement of parasites and vectors across the border from Mozambique may promote malaria transmission in eastern Zimbabwe. Using a malaria risk map and controlling for ecological factors, we will quantify the increased risk due to ecological factors and health seeking behaviors on the Mozambique border in Mutasa District, Zimbabwe, providing a deeper understanding of cross-border malaria transmission.

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DEFINING THE MICRO-EPIDEMIOLOGY OF MALARIA

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Malaria risk varies considerably over fine spatial scales but this 'micro-epidemiology' is not well understood. A systematic review and meta-analysis was conducted to identify factors that explain micro-epidemiological variation in malaria risk and define the scope, theory and methods for malaria micro-epidemiology. PuBMed, ISI Web of Knowledge and LILACS databases were searched for studies assessing variation in malaria risk between individuals or households within villages or between neighbouring villages. We included 51 of 738 studies screened that investigate demographic, social, environmental and epidemiological risk factors. Most studies investigated environmental risk factors for malaria, of which proximity to breeding sites and housing structure most frequently explained variation in risk. Social characteristics beyond bed net use were not widely considered, though mobility patterns and access to health care were frequently associated with malaria risk. There is limited evidence that crude estimates of the effects of environmental factors are confounded by social and epidemiological characteristics, including village population size and clinical and genetic characteristics, yet these variables are not included in most studies. There was substantial heterogeneity in effect estimates, including by study design, transmission context, exposure classification and analysis level, as there was only partial overlap between factors associated with malaria risk at individual compared to cluster or village level. Pooled estimates could therefore not be produced. Instead, a causal framework for the relationships between different malaria risk factors associated at micro-epidemiological scales was developed. In conclusion, micro-epidemiological studies should measure social, epidemiological and environmental factors consistently associated with fine scale variation in malaria risk. Control of confounding and multilevel analysis could be improved through use of causal frameworks for design and analysis of micro-epidemiological studies of malaria.

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CHARACTERIZING THE ASYMPTOMATIC AND SUBMICROSCOPIC MALARIA RESERVOIR IN SOUTHERN ZAMBIA: ASSOCIATED RISK FACTORS AND GAMETOCYTE PREVALENCE

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To achieve and sustain malaria elimination, identification and treatment of the chronically infected, asymptomatic reservoir is critical. Such individuals are not likely to seek medical care yet can harbor gametocytes and serve as reservoirs for transmission. Characteristics of asymptomatic individuals infected with *Plasmodium falciparum* were evaluated in Choma District, southern Zambia where malaria transmission has declined dramatically over the past decade. Households were randomly selected for participation in community-based, cross-sectional surveys between 2008 and 2013. Questionnaires were administered to collect information on age, sex, recent history of malaria symptoms and recent anti-malarial medication use. Asymptomatic malaria was defined as the absence of fever (tympanic temperature ≥38°C) on the visit day or no self-reported fever with chills during the last 48 hours. Blood samples were collected by finger prick for Pfhrp2-based rapid diagnostic tests (RDT), blood smears and dried blood spots (DBS). DNA was extracted from the DBS and a cytb-targeted nested PCR (nPCR) and a Pfs25 RT-nPCR were performed to detect malaria parasites and gametocytes, respectively. Of 4,101 participants with complete data, 99 (2.4%) were positive by RDT or nPCR and 98% (n=97) of them lacked visible parasites by microscopy. Seventy-four % of these malaria cases (n=73) were classified as asymptomatic and 52% (n=38) of the asymptomatic cases were RDT negative but nPCR positive. Compared to RDT and nPCR negative individuals, asymptomatic, RDT negative and nPCR positive individuals were more likely to be male (p=0.004) and all were above 5 years of age. The prevalence of gametocytemia by RT-nPCR was higher among participants who were RDT and nPCR positive (33%) or RDT negative but nPCR positive (24%) than those who were RDT and nPCR negative (1.1%; p<0.0005). In areas of declining malaria transmission where the majority of infected individuals are RDT negative, more sensitive screening tools or focal drug administration strategies are needed for further reduce malaria transmission and achieve malaria elimination.

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RETURNING TO THE PROBLEM OF MALARIA IN CHILDREN UNDER FIVE IN LIBERIA

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Prior to the Ebola virus (EVD) epidemic, Liberia was recovering from a prolonged civil war that had decimated the health care system. Malaria was reported to be the leading cause of inpatient admission and death in children under 5 years of age. Determining the cause of acute disease in endemic regions is often complicated by the presence of premonition - 32% of children in Liberia test positive for malaria and are asymptomatic.

Thus, other causes of acute febrile illness may mimic malaria and remain untreated. We conducted a prospective, hospital-based study of children under 5 years who presented to JFK Medical Center in Monrovia with presumed malaria from June 2013-May 2014. Clinical data was obtained on admission and discharge. Malaria diagnosis was confirmed by microscopy and/or malaria rapid diagnostic test. Children were treated for malaria using national treatment guidelines. 351 children who were admitted to JFK and treated for severe malaria agreed to participate in the study. Of this cohort, 34% were previously admitted to the hospital from 1-4 times for treatment of malaria. 49% of this cohort met the case definition of severe malaria (confirmed malaria infection with resolution of symptoms and parasitemia by day 3). For these patients, the most common presenting symptoms included fever (100%) for an average of 3.8 days prior to presentation, headache (95%), prostration (80%), cough (68%), seizures (33%), diarrhea (30%) and respiratory distress (25%).. 44% of patients had anemia on admission with an average hemoglobin level of 9.8 mg/dl. 26% of these patients hospitalized with presumed severe malaria tested negative for malaria. The most common presenting complaints in this group were; cough (47%), headache (27%), prostration (20%), diarrhea (16%) and respiratory distress (4%). In conclusion, this pilot study clearly demonstrates that children in Liberia suffer from more than malaria and should be clinically assessed and treated for other febrile illnesses. Future studies will include defining the landscape of febrile illness in children in Liberia.

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POPULATION-BASED MALARIA SURVEILLANCE BY HEALTHCARE WORKERS IN THE PROVINCES OF HAUT KATANGA AND LUALABA IN THE DRC

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In the Democratic Republic of the Congo (DRC), malaria is reported to be the primary cause of morbidity and mortality, estimated to account for >40% of outpatient visits and attributable mortality of 40% in children under 5. However, health information systems are not widely available and consequently the accuracy of field data is limited. A public-private partnership was established to implement a malaria case management and surveillance system in Haut Katanga and Lualaba provinces. Over 250 public primary healthcare workers and managers are being equipped with Fionet, an integrated system with point-of-care devices to guide case management and analyze RDT test results combined with remote oversight. The implementation of Fionet commenced in January 2016 and will be fully implemented by November 2016. As of March 2016, there are 150 healthcare workers covering >20% of public health facilities in the two provinces that are operational with Fionet. 6,780 malaria RDTs have been processed with Fionet, which ensures quality processing and automated interpretation, and uploaded to Fionet cloud for monitoring, quality control, analysis and reporting. In febrile children under 5 years of age, we observe an 80.6% positivity rate, which is one the highest levels reported globally. Additionally, 62% of patients over 5 years of age and 56% of pregnant women tested positive for malaria. Patient information is also routinely collected on Fionet, for example, only half of the patients' report having a bed net in their household. Fionet has demonstrated feasibility and usability in the hands of healthcare workers in the field. The implementation at scale will provide accurate, population-based, real-time data on malaria prevalence with the ability to allocate resources and monitor outbreaks. Field data collected using the system can be integrated with databases (e.g. DHIS2) and utilized for public health decision-making and research.

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EVALUATION OF SEROLOGICAL BIOMARKERS OF *PLASMODIUM VIVAX* AND *P. FALCIPARUM* TRANSMISSION IN THE SOLOMON ISLANDS

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In the Solomon Islands, malaria transmission is low, highly heterogeneous, and dominated by *Plasmodium vivax* (*Pv*), but has pockets of persistent *P. falciparum* (*Pf*). This study utilized a cross-sectional serological survey of 2000 individuals of all ages from five geographical regions of Ngella Province of varying transmission levels, analyzing antibody responses to 13 *Pf* and 8 *Pv* antigens using a bead-array immunoassay. Methods were developed to assess optimal cutoffs for serological positivity, cross-reactivity between and Pv and Pf antibodies, seroconversion rates, and correlations between seropositivity and infection rates in the different transmission zones to identify the most promising antigens as biomarkers of transmission. Significant correlations were found for four antigens: *Pf* CelTOS (p=0.036, r=0.90), *Pf* MSP2 (p=0.026, r=0.92), *Pv* DBP-AH (p=0.045, r=0.89), and *Pv* DBP-P (p=0.019, r=0.94). The results show that antibody responses to these four antigens are promising biomarkers of malaria transmission levels and suggest that variants of the *Pv* Duffy Binding Protein are particularly indicative of malaria exposure. Additional analysis is underway to confirm these initial findings and to identify additional optimal serological biomarkers of malaria transmission.

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EPIDEMIOLOGY OF CHRONIC ASYMPTOMATIC *PLASMODIUM FALCIPARUM* INFECTIONS AMONG ALL AGES IN AN AREA WITH SEASONAL MALARIA TRANSMISSION IN BONGO DISTRICT, GHANA

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Despite efforts to control and eliminate *Plasmodium falciparum*, malaria still remains a major public health concern. Exposure to antigenically diverse *P. falciparum* isolates at a young age leads to the acquisition of protective immunity and the development of chronic asymptomatic malaria infections in endemic areas. Understanding the role asymptomatic infections play in sustaining the reservoir of infection needs to be examined so that countries can shift towards malaria elimination. This research describes a longitudinal cohort (N=2,000) study designed to evaluate the reservoir of asymptomatic *P. falciparum* infections among all ages in an area with seasonal transmission in Bongo District, Ghana. Using different methods for parasite detection we evaluated how age, seasonality, spatial location and other factors affect the epidemiology of asymptomatic malaria at the end of the 2012/13 wet and dry seasons. Asymptomatic *P. falciparum* prevalence by microscopy decreased significantly from 42.5% at the end of the wet to 27.5% at the end of the dry season ($p < 0.001$). Using the 18S rRNA nPCR, all microscopy negative samples were further screened for submicroscopic infections. Resulting prevalence of submicroscopic infections also decreased significantly, with 55.4% and 20.7% at the end of the wet and dry seasons respectively ($p < 0.001$). Combining detection methods, 74.4% of the population in the wet and 42.5% in the dry season had evidence of an active *P. falciparum*

infection. Interestingly in those >20 years of age, we found evidence of infection in 64.3% of the population in the wet and 27.0% in the dry season. Using the combination of microscopy and PCR we have shown that the asymptomatic reservoir peaks at the end of the wet season and that infections in all age groups contribute to maintaining the reservoir of malaria infection. These results suggest that if elimination is to succeed, interventions will need to target not just children but all asymptomatic *P. falciparum* infections and be implemented towards the end of the dry season in this area of West Africa.

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DEMOGRAPHIC AND CLINICAL PROFILES OF *PLASMODIUM FALCIPARUM AND P. VIVAX* PATIENTS AT A TERTIARY CARE CENTER IN SOUTHWESTERN INDIA

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India is a highly heterogeneous country, comprising more than 1.2 billion people, 2000 ethnic groups and 22 official languages. On the Indian subcontinent, there are more than 500 million people at risk for malaria, with reports of up to two million cases and 50,000 deaths per year. In contrast with Africa, malaria transmission is more limited, severe malaria disease is more frequently detected in adolescents and adults, and a substantial proportion of cases are infected with *P. vivax* rather than the traditionally more-virulent *P. falciparum* in India. Expansive studies on determinants of severe disease and mortality among malaria-positive patients are fewer and smaller in scope than in Africa or SE Asia. The present study describes the population served by Goa Medical College (GMC) and the demographic, diagnostic and clinical characteristics of malaria-positive study participants enrolled to-date at the Centre's principal research site. A total of 74,571 febrile individuals presented to GMC between January 2012 and December 2015 and were tested for malaria. Of those, 6,277 (8.4 %) were determined to be positive for malaria infection. Over four years of passive surveillance, the number of malaria-positive cases presenting to GMC steadily and significantly increased, from 873 cases in 2012 to 2,263 cases in 2015. While, a critical component of modern improvements in patient care are to meld clinical care, research, and treatment activities with the powerful potential of basic science to untangle variables that may contribute to susceptibility, pathogenesis, and resistance in malaria, this is not always possible on a large scale. Based on extensive statistical analysis of our patient group, the present study reveals three potentially valuable, simple prognostic indicators of disease severity in India among malaria-positive patients: increasing age, high fever and anaemia (others were ruled out). The predictive indicators may be employed by clinicians at GMC and in similar resource-limited settings when making hospital admissions decisions.

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CONSERVED SEQUENCE MOTIFS IN PLACENTAL MALARIA VACCINE CANDIDATE VAR2CSA DESPITE LARGE OVERALL SEQUENCE DIVERSITY

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VAR2CSA, a member of the *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) family, mediates the binding of *P. falciparum*-infected erythrocytes to chondroitin sulfate A, and is a key protein in the pathogenesis of placental malaria. VAR2CSA is a leading vaccine candidate against placental malaria, as it is a target of naturally acquired immunity to the disease. However, antigenic diversity presents a significant challenge to the development of a VAR2CSA-based vaccine. A broadly effective vaccine that overcomes strain specificity will likely require more than one allele of var2csa. To evaluate the possibility of regional differences in vaccine efficacy, we investigated whether sequence similarity among var2csa alleles is related to their geographic origin. We analyzed 90 var2csa allelic sequences from 12, 43, and 7 samples from West Africa, East Africa, and a published dataset reflecting global diversity, respectively. The sequences from East Africa were generated using Pacific Biosciences (PacBio) amplicon sequencing, whereas those from West Africa were assembled from a combination of PacBio and Illumina whole genome sequence data. We analyzed patterns of similarity, based both on overall and partial sequences, as well as on k-mer composition. Our preliminary results show that VAR2CSA proteins are highly diverse (mean amino acid sequence similarity of 75%) and sequences did not cluster by geographic origin. However, we identified some conserved motifs in DBLepam4 and DBLepam5 among parasites collected from distant geographic regions. These findings support the possibility of developing a broadly protective VAR2CSA-based vaccine from a limited number of strains. We are extending our analysis with additional sequences from Southeast Asian isolates.

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INDEPENDENT ORIGIN AND GLOBAL DISTRIBUTION OF DISTINCT *PLASMODIUM VIVAX* DUFFY-BINDING PROTEIN GENE DUPLICATIONS

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Plasmodium vivax causes the majority of malaria episodes outside Africa, but remains a relatively understudied pathogen. The pathology of *P. vivax* infection depends critically on the parasite's ability to recognize and invade human erythrocytes. This invasion process involves an interaction between

P. vivax Duffy-binding protein (PvDBP) in merozoites and the Duffy antigen receptor for chemokines (DARC) on the erythrocyte surface. Whole-genome sequencing of clinical isolates recently established that some *P. vivax* genomes contain two copies of the PvDBP gene. The frequency of this duplication is particularly high in Madagascar, where there is also evidence for *P. vivax* infection in DARC-negative individuals. The functional significance and global prevalence of this duplication, and whether there are other copy number variants at the PvDBP locus, is unknown. Using whole-genome sequencing and PCR to study the PvDBP locus in *P. vivax* clinical isolates, we found that PvDBP duplication is widespread in Cambodia. The boundaries of the Cambodian PvDBP duplication differ from those previously identified in Madagascar, meaning that current molecular assays were unable to detect it. The Cambodian PvDBP duplication did not associate with parasite density or DARC genotype, and ranged in prevalence from 20% to 38% over four annual transmission seasons in Cambodia. This duplication was also present in *P. vivax* isolates from Brazil and Ethiopia, but not India. PvDBP duplications are much more widespread and complex than previously thought, and at least two distinct duplications are circulating globally. The same duplication boundaries were identified in parasites from three continents, and were found at high prevalence in human populations where DARC-negativity is essentially absent. It is therefore unlikely that PvDBP duplication is associated with infection of DARC-negative individuals, but functional tests will be required to confirm this hypothesis.

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INTERACTIONS AND COMPETITIVE GROWTH WITHIN MIXED INFECTIONS OF *PLASMODIUM FALCIPARUM*

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Infections by multiple genetically distinct *Plasmodium falciparum* parasites are common in cases of human malaria. The dynamic interactions of parasites in mixed infections include competition between co-infecting strains and potential for selection of parasites with fitness advantages. With emerging artemisinin resistance, it is essential to understand the relative fitness of parasites exhibiting the delayed clearance phenotype that could influence their spread in populations. Although artemisinin resistance has been associated with the *pfkelch13* gene, the *in vivo* delayed clearance phenotype does not have a corresponding *in vitro* IC_{50} shift; furthermore, resistant parasites that lack *pfkelch13* mutations have not been well characterized. The relative fitness of parasites displaying delayed clearance to artemisinin, both with and without *pfkelch13* mutations, is unknown, emphasizing the need for analyses of fitness costs and benefits of resistance mutations. In this research, pair wise competition assays were used to ascertain fitness of mutations associated with delayed clearance to artemisinin treatment. Slow clearance parasite isolates from Southeast Asia, with and without *pfkelch13* mutations, were evaluated. Results indicate a range of relative fitness phenotypes associated with different mutations. Further experiments are in progress to implement competitions of these isolates in the presence of low level artemisinin drugs to elucidate which resistance associated mutations provide the most fitness for parasites to proliferate under drug pressure within mixed infections.

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MULTIPLEX BARCODED NEXT-GENERATION SEQUENCING OF MULTICLONAL *PLASMODIUM FALCIPARUM* GENOTYPES

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Large-scale molecular epidemiologic studies of *Plasmodium falciparum* parasites can investigate parasite biology and transmission, identify and predict the spread of drug resistance, and assist in the evaluation of vaccine candidates. The polyclonal nature of most infections in high transmission settings undermines many traditional genotyping approaches. Next-generation sequencing approaches to parasite genotyping may allow sensitive detection of minority variants, disaggregation of complex parasite mixtures and scalable processing of large samples sets. Therefore, we designed, validated, and applied to field parasites a new approach to parasite genotyping that leverages next-generation sequencing of individually barcoded samples in a highly scalable and multiplex manner. We utilize variant barcodes, invariant linker sequences and modular template specific primers in such a way as to allow for the simultaneous generation of high-dimensional sequencing data of multiple gene targets. The modularity of this approach permits a cost-effective and easily reproducible way to query many genes without experimental redesign. In practice, this approach generates large numbers of high quality reads in a manner that is robust to different sequencing technologies including both Ion Torrent and Illumina MiSeq. In mixtures of reference parasite genomes, we qualitatively and quantitatively detected unique haplotypes comprising 0.1% of polyclonal infection. We demonstrate concordance of the outcomes of this method compared to traditional Sanger sequencing and pooled next-generation sequencing. Finally, we applied this genotyping approach to fresh parasites collected in Western Kenya in order to rapidly obtain parasites genotypes at three unlinked loci. In summary, we present a rapid, scalable and flexible method for genotyping individual *P. falciparum* parasites that further enable molecular epidemiologic studies of parasite evolution, population structure and transmission.

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THE DIVERSITY OF RNAS EXPRESSED IN *PLASMODIUM VIVAX*

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The biology of the widespread human malaria parasite, *Plasmodium vivax*, remains largely unknown due to a lack of a robust *in vitro* culture system. Here, we used stranded RNA-sequencing technologies paired with depletion of highly expressed transcripts from the host (i.e. globin mRNAs and rRNAs) for assessing global transcriptome changes in the host and parasite during infection and treatment. From 100uL of patient blood, we isolated RNAs and sequenced >50 million paired end reads from three patients. 15-25% of the reads aligned to the *P. vivax* genome sequence, as a result of >80% reduction in human rRNAs and globin mRNAs. Using this large amount of sequence data, we *de novo* assembled all RNA transcripts expressed by intraerythrocytic *P. vivax* parasites. Many of our transcripts coincide with annotated protein-coding genes, though a very high number of genes have misannotated 5' and 3'UTRs that often include unannotated introns likely involved in gene regulation. Additionally, we identified 1,388 genes with multiple isoforms as a result of alternative splicing, intron retention, and alternative transcriptional start and stop sites. 5,207 assembled transcripts had no coding potential and are likely noncoding RNAs. Because our data can separate reads that originate from different strands of RNA, we can assess that a large number of these noncoding reads are antisense RNAs for coding genes, while the rest are a mixture of small RNAs and intergenic long noncoding RNAs. Finally, as a result of high read coverage, we were able to additionally find >4,800 polymorphisms in each patient sample, data that can help us to determine the complexity of infection and how different polymorphisms affect transcription. Together, our study reveals the diversity and complexity of RNAs expressed by intraerythrocytic *P. vivax* parasites and show that stranded RNA-seq is a robust method to study host/parasite interactions using patient samples.

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EXCEPTIONALLY LONG-RANGE HAPLOTYPES IN *PLASMODIUM FALCIPARUM* CHROMOSOME 6 MAINTAINED IN *AN* ENDEMIC AFRICAN POPULATION

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Previous genome-wide analyses of single nucleotide variation in *Plasmodium falciparum* identified evidence of an extended haplotype region on chromosome 6 in West Africa, suggesting recent positive selection. Such a pattern is not seen in samples from East Africa or South East Asia, so it could be marking a selective process particular to West Africa. Analyses of the haplotype structure in samples taken at different times could give clues to possible causes of selection. This study investigates chromosome 6 extended haplotypes in the Gambia by analysing alleles at multiple microsatellite loci using genome sequence data previously obtained from clinical isolates collected in 2008, followed by genotyping 13 loci in 405 isolates from 1991, 2008 and 2014. Multiple long haplotypes were evident in the population sample, and a region of high linkage disequilibrium was shown to span ~200 kilobases (Kb), with a core region of ~70 Kb having the most intact haplotype structure. Two of the haplotypes were detected in samples from 1991, which predates the time when chloroquine and antifolate resistance alleles became common locally, and these haplotypes were still present in 2014. The occurrence of several long haplotypes at intermediate frequencies suggests an unusual mode of selection in chromosome 6, possibly combined with recombination suppression on specific haplotypes. Such selection apparently occurred before the emergence of known antimalarial drug resistance alleles, and could be due to effects of other drugs or unknown processes that have long been operating in this endemic region.

1596

DE NOVO VARIANT CALLING TO RESOLVE TRANSMISSION DYNAMICS WITHIN CLONAL *PLASMODIUM FALCIPARUM* SAMPLES: A CRUCIAL TOOL FOR THE MALARIA 'ENDGAME'

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Genetic studies have provided an increasingly clear view of malaria transmission; genotyping a small number of loci can allow us to study changes in transmission dynamics and relatedness in response to malaria control. Yet the genetic epidemiology of malaria lags far behind other pathogens. In bacterial or viral pathogens the higher mutation rates allow de novo variation to be used. With the *Plasmodium falciparum* genome, however, SNPs do not show sufficiently high mutation rates to allow us to distinguish between 100% related parasites. Conversely short tandem repeat (STR) loci, which mutate many orders of magnitude faster, are difficult to genotype from short-read sequence. Recent work at the Broad Institute - improved library preparation, read lengths, and methods of genotyping - now enable this kind of study to be undertaken. Applying these methods to *P. falciparum*, we show that INDEL and STR variation can now be called with greatly increased accuracy even in low-complexity regions of the *P. falciparum* genome, affording newfound access to significant amounts of de novo variation. We have employed these approaches to examine a set of parasites from Thies, Senegal that have not outbred and indistinguishable using current genotyping approaches. Using de novo variants only we have derived phylogenies and transmission networks for these parasites. As we move towards low-transmission or 'pre-elimination' settings, in which highly-related parasites are the norm, we propose a framework to examine de novo mutation as a crucial tool for the 'endgame' of malaria eradication. By using derived mutation we add a valuable temporal dimension to genomic epidemiology; we will show how examining substitution rates in clinical sample sets can distinguish between different modes of transmission - potentially allowing us to identify superspreaders within transmission chains.

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ELUCIDATION OF THE DIVERGENT APICOPLAST GENOMES OF *PLASMODIUM OVALE CURTISI* AND *P. OVALE WALLIKERI*

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Whole genome sequencing was attempted on parasite DNA extracted from the peripheral blood of malaria patients infected with *Plasmodium ovale wallikeri*. A full-length contig of the apicoplast genome was successfully assembled and this was compared to published sequences from the *P. ovale curtisi* apicoplast genome. Observed dimorphic regions are being confirmed in archived DNA from multiple isolates of both species. The potential for an apicoplast-mitochondrial barcode of defined polymorphisms that perfectly discriminates these two closely related parasite species will be considered.

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WHOLE-GENOME PROFILING OF DIFFERENTIALLY EXPRESSED GENES IN CHILDREN WITH MALARIAL ANEMIA

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Application of whole genome expression profiling is a useful approach for identifying important gene pathways associated with disease outcomes, especially in a multifocal disease such as falciparum malaria. In high transmission regions, malaria commonly manifests as severe malarial anemia [SMA, hemoglobin (Hb)<5.0 g/dL], primarily in infants and young children. Currently, the molecular mechanisms that condition the development of SMA are largely undefined. Therefore, use of unbiased transcriptomics is suitable in young children with SMA where sample volume is limited to perform individual gene quantification. Malarious children (n=1218, aged 3-36 mos) were stratified based on disease severity into 'polarized' extremes of non-SMA (Hb, 8.0-10.9g/dL, n=532) and SMA (n=228) groups after exluding children's with co-infections (bacteremia and HIV-1) and hemoglobinopathies (SSD, G6PD deficient and α-thalassemia). RNA was isolated from leukocytes collected on first hospital enrollment to the study prior to treatment interventions. Based on RNA quality checks, 72 samples (non-SMA, n=51; SMA, n=21) were selected for transcriptomics analysis. Gene expression analysis was performed using the Illumina® HumanHT-12 beadchip covering 47,231 transcripts specific to 19,185 genes. Data were analyzed through step-wise procedures to exclude transcripts identified as having an "absent" expression. A second quality control filter removed transcripts with low signal values, resulting in 3,981 genes. Transcripts with ≥1.5-fold change in SMA relative to non-SMA group (P<0.05) were 629 genes; 597 upregulated and 32 downregulated. To infer biological significance, we generated relational pathways, resulting in 30 networks. The networks that showed highest significance (P=3.53x10-19) were enriched for immune response, signal transduction and hematopoiesis genes. Additionally, validation of selected genes [HSPA1A (n=89), IL-18 (n=77) and COX2 (n=23)] showed an

identical trend. In summary, transcriptomic arrays identified both novel and known genes/gene pathways that are important for the host immune response to malaria infection.

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ANALYSIS OF MULTIPLICITY OF ETHIOPIAN *PLASMODIUM VIVAX* INFECTIONS AND RELAPSE PATTERNS USING PVMSP1 AMPLICON DEEP SEQUENCING

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Parasite genetic diversity and multiplicity of infection (MOI) affect clinical outcomes, response to drug treatment and naturally acquired or vaccine-induced immunity. Based on microsatellite and merozoite surface protein (MSP) markers, a number of studies have reported on MOI and the frequency of multiclonal infections in Plasmodium parasites. However, traditional methods often underestimate the MOI and the frequency of multiclonal infections due to technical sensitivity and specificity. Next-generation sequencing techniques provide a novel opportunity to study parasite diversity. In this study, we conduct amplicon deep sequencing of PvMSP1 to determine MOI and detect the relapse pattern of *Plasmodium vivax* from Southwestern Ethiopia. A total of 139 *P. vivax* dry blood samples were pyro-sequenced on a 422 bp fragment of PvMSP1 amplicon, yielding a total of 231 haplotypes. The average of MOI was 4.68, ranging from 2 to 14 clones in a single individual. However, using 3 microsatellite markers, an average of MOI=2.64 was detected with only 1-5 clones in a single subject. Four (80%) out of 5 subjects with recurrent vivax malaria were found to be relapse 44-65 days after chloroquine treatment. Significantly different MOIs were found among age groups, locations, and transmission seasons as well as between symptomatic and asymptomatic samples. Significantly higher MOI was found in clinic samples. Young children and old adults showed a higher MOI than that of older children. These results suggest that *P. vivax* multiclonal infections were common together with high proportions of relapse in Ethiopia. This study has important implication for the provision of primaquine to prevent relapse, anti-relapsing interventions and eliminating malaria in the low transmission areas.

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IMPROVED, HIGH-RESOLUTION SINGLE-CELL GENOMIC PROFILING OF HUMAN MALARIA PARASITES

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In many cases, malaria patients suffer from complex infections caused by multiple parasite lineages. The prevalence of, and interactions between, unique parasite genotypes within such multiple genotype infections are wholly unknown, complicating efforts to understand the spread of drug resistance, genetic recombination, and transmission to mosquitoes. For a first look at component genotypes within complex infections, we previously developed a single-cell genomics platform where individual parasites were briefly cultured *ex vivo*, captured by flow cytometry and whole-genome amplified prior to deep sequencing. Our analyses showed that coverage of the genome was highly variable, limiting the construction of complete haplotypes. We have since further optimized our protocol, focusing our analysis on late-stage parasites, which contain a higher amount of DNA template, by increasing the time of culture and utilizing restrictive flow cytometry gating. Analysis of these replicating parasites yielded superior genomic data in terms of the rate of successful amplification, the fraction of reads mapped to the parasite genome (purity), and genome-wide coverage. With broad haplotype blocks in hand, it is now feasible to obtain a comprehensive portrait of genetic variation in each cell. Here, we report the analysis of several dozen individual *Plasmodium falciparum* genomes collected from patients in a region of high malaria transmission. These improvements enable characterization of the genetic diversity in malaria infections at unprecedented resolution and scale.

1601

DISTINCT ARCHITECTURE OF *PLASMODIUM FALCIPARUM* POPULATIONS FROM SOUTH ASIA, WITH COUNTRY-LEVEL CLUSTERING OF GENOMIC RELATIONSHIPS

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India records about two to ten million cases of malaria every year, with about 50,000 deaths per year. Compared to the heavy emphasis of malaria research in Africa and in Southeast Asia, much less is known about genomic and phenotypic properties of parasites from the Indian subcontinent and such omissions need attention since *Plasmodium falciparum* isolates differ genetically as well as by phenotypes relative to their geographic origins. Previous genome-wide comparisons have analyzed stratification in global parasite populations across different continents. However, these studies have not included Indian isolates in the past. Here, we examine whole genome sequences from 23 Indian parasite isolates and their relationships with hundreds of other isolates from around the globe. Our analysis provides a rich collection of over 360,000 high quality variants. The entire collection of these variants was used to calculate a nucleotide distance estimate between each pair of global isolates. Principle coordinate analysis showed that parasite isolates segregate based on geographic locations, where an entire cluster can be classified as originating from a single continent. Removing the highly variable var genes from all the genomes prior to estimating the pair-wise distances eliminates many of the sequencing and alignment errors and reveals an even higher resolution geographic segregation. Surprisingly, Indian isolates segregate into a unique cluster widely separated even from other South Asian isolates. Nearest neighbors to Indian isolates are Bangladesh followed by other South-East Asian countries. This, therefore, reveals a unique place for India in the world malaria map. Monitoring of global malaria elimination strategies as well as global parasite evolution will have to include India due to the unique history of these parasites and their strategic position between Southeast Asia and Africa.

1602

EVALUATING THE INFORMATION VALUE OF PARASITE GENOMICS FOR MALARIA ELIMINATION

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As countries push towards malaria elimination, there is an increasing need to understand the disease transmission network and particularly the dynamics of residual foci. Sequencing parasite genomes has the potential to distinguish between local and imported cases by source, to assist in categorizing areas with varying capacities for transmission and their relative connectivity, and to validate the relevant time and space scales that define effectively disconnected regions. Using a dynamical model that tracks full parasite genomes of indiviudal *Plasmodium*

falciparum infections, we demonstrate the potential information value of different sequencing technologies and sampling frames to address these operationally critical questions.

1603

ABSENCE OF *IN VIVO* SELECTION OF K13 POLYMORPHISMS AFTER ARTEMETHER LUMEFANTRINE TREATMENT IN UGANDA

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In Southeast Asia, the *Plasmodium falciparum (Pf) kelch13* (PF3D7_1343700) gene constitutes a useful molecular marker for artemisinin resistance surveillance. Mutations in the *Pfkelch13* are known to be involved in the development of delay of parasite clearance after artemisinin treatment. This delayed clearance has also been significantly associated with the following six particular SNPs in *Pf; ferredoxin (fd), apicoplast ribosomal protein S10 (arps10), multiple resistance protein 2+(mdr2), chloroquine resistance transporter (crt), phosphoinositide-binding protein (pibp)* and *protein phosphatase* (*pph)* genes in South East Asia. Individual treatment would select resistant parasites in the human body, namely *in vivo* selection. Currently, there's a paucity of data about in-vivo selection of the above mentioned mutations. We conducted an artemether-lumefantrine (AL) follow-up study in Uganda, in which genotypes in *Pfkelch13* and six SNPs were compared before drug administration and in all recurrent parasites during a follow-up period of 28 days. We found that AL treatment was very effective with PCR adjusted efficacy of 95.1%. Only three cases showed late clinical failures. Among a total of 161 isolates before AL treatment, almost all (96.8%) had wild type alleles in *Pfkelch13*. Similarly, only wild type alleles were observed in *fd, arps10, mdr2, pibp* and *pph* genes. Mixed alleles (wild and mutant) were observed in 2.3% of isolates in *crt*. In all follow-up cases, presence of parasites was molecularly confirmed and 21 positive results were obtained. All these isolates harbored wild type alleles in *Pfkelch13* and the six genes. These results suggest that very few isolates were observed after AL treatment in Gulu Northern Uganda, but this may not be the case because of the potential selection of mutant alleles in the genes that are associated with artemisinin resistance in Southeast Asia.

1604

EUPATHDB: A POWERFUL EUKARYOTIC PATHOGEN GENOMIC AND FUNCTIONAL GENOMIC DATA MINING RESOURCE

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The Eukaryotic Pathogen Database (EuPathDB, http://eupathdb.org) is a free, online data mining resource that facilitates the discovery of meaningful biological relationships from large volumes of data by integrating pre-analyzed omics data with advanced search capabilities, data visualization and analysis tools. EuPathDB supports over 170 organisms within Amoebazoa, Apicomplexa, Chromerida, Diplomadida, Trichomonadida, Kinetoplastida and numerous phyla of oomycetes and fungi. For these organisms, EuPathDB integrates a wide range of data including genome sequence and annotation, transcriptomics, proteomics, epigenomics, metabolomics, population resequencing clinical and field isolates, and data that inform host-pathogen interactions. Data are analyzed using standard workflows and an in-house analysis pipeline generates data including domain predictions, orthology profiles across all genomes and GO term associations. Our unique strategies system offers over 100 structured searches that query the pre-computed data. Individual search results can be combined into strategies that easily merge evidence from diverse data types and across organisms. Easily accessible tools enhance the search strategy system and include dynamic data visualization, comparative genome analysis, population genetics tools, and functional or pathway enrichment. Forthcoming new tools and functionalities include a private user work-space for primary data analysis, functional analysis tools for result summarization, genome browser and query improvements. This comprehensive resource places the power of bioinformatics with the entire scientific community in support of hypothesis driven research. EuPathDB's active user support offers an email help desk (help@eupathdb.org), social media, a You Tube channel with tutorials and a worldwide program of workshops.

1605

THE BIOLOGICAL FUNCTION OF ANTIBODIES INDUCED BY THE RTS,S/AS01 MALARIA VACCINE CANDIDATE IS DETERMINED BY THEIR FINE SPECIFICITY

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Recent vaccine studies suggest that the magnitude of an antibody response is often insufficient to explain efficacy, suggesting that characteristics regarding the quality of the antibody response, such as its fine-specificity and functional activity, may play a major role in protection. Previous studies of the lead malaria vaccine candidate, RTS,S, have shown that circumsporozoite protein (CSP)-specific antibodies and $CD4^+$ T cell responses are associated with protection, however the role of fine specificity and biological function of CSP-specific antibodies remains to be elucidated. Here, we addressed the relationship between fine specificity, opsonization-dependent phagocytic activity, and protection in RTS,S-induced antibodies. We developed a new method for measuring the phagocytic activity mediated by CSP-specific antibodies and applied it to samples from a completed phase 2 RTS,S/AS01 clinical trial. We also assessed the fine-specificity of the antibody response using ELISA against three antigen constructs of CSP: the central repeat region, the C-terminal domain, and the full-length protein. We carried out multi-parameter analysis of phagocytic activity and fine-specificity data across to identify potential correlates of protection in RTS,S. We found that phagocytic activity was correlated with full-length CSP and C-terminal specific antibody titers, but not to repeat region antibody titers. When expressing the phagocytic activity as 'opsonization index', a relative measure that normalizes phagocytic activity with CS antibody titers, we found, surprisingly, that protected subjects had a significantly lower opsonization index than non-protected subjects. The data suggest that the opsonization is a surrogate marker of protection induced by the RTS,S/AS01 vaccine and determined how antibody fine-specificity is linked to opsonization activity. Our findings suggest that the role of opsonization in protection

in the RTS,S vaccine may be more complex than previously thought, and demonstrate how integrating multiple immune measures can provide insight into underlying mechanisms of immunity and protection.

1606

PLACENTAL MALARIA IS ASSOCIATED WITH ALTERED FETAL CYTOKINE PROFILES

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Malaria during pregnancy threatens the health of mothers and newborns and may have long-lasting consequences on infant health. Our previous work shows that placental malaria is associated with increased risk of malaria in the infant. We hypothesize that this is due to priming of the fetal immune system toward immunoregulatory responses as a consequence of maternal malaria infection. We collected cord blood serum from children born to mothers with detailed antenatal histories and followed a subset of these children through the first year of life, collecting serum at 12 months of age. We used multiplexed electrochemiluminescent immunoassays (Meso Scale Discovery) to measure 11 cytokines (IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-13, IFNγ, TNFα, TGFβ and CRP). We analyzed cord serum from 26 infants born to mothers with no malaria during pregnancy, 26 born to mothers with peripheral malaria, 87 born to mothers with placental malaria, and 14 North American control infants never exposed to malaria. We observed that children born to mothers with chronic placental malaria had significantly elevated levels of TNFα (a pro-inflammatory cytokine), IL-10 (an immunoregulatory cytokine) and CRP (a marker of inflammation) at the time of birth as compared to children born to mothers with peripheral malaria during pregnancy ($p=0.003$, $p=0.001$, $p=0.014$, respectively), no malaria during pregnancy ($p=0.003$, $p=0.037$, $p=0.006$, respectively) or North American controls ($p=0.002$, $p<0.001$, $p=0.045$, respectively). Cytokine levels normalized by one year of age. We propose a model in which placental malaria causes chronic *in utero* inflammation with compensatory production of IL-10 and induction of T regulatory cells (Tregs). After birth, cytokine levels normalize, but Tregs are maintained preventing effective immune responses to malaria and resulting in increased risk of malaria during infancy. We are currently conducting flow cytometric studies on cord blood to further explore this hypothesis. Our results will inform the design and implementation of prenatal interventions to protect the health of pregnant women, newborns and infants from malaria.

1607

PLASMODIUM FALCIPARUM INFECTION AND VACCINE RESPONSES: SHOULD WE TREAT PRESUMPTIVELY?

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Individuals with malaria may have blunted immune responses to some vaccines, suggesting that there is an active immune suppression or immunomodulation during infection. How and which vaccines are impacted by clinical malaria or asymptomatic parasitemia is not completely clear, nor is whether the impacts are sufficient to recommend delaying or presumptively treating individuals prior to routine vaccinations or in malaria vaccine trials. In a series of studies, we are examining the impact of malaria on immune cell function and on vaccine responses in cohorts of adults at various study sites in Mali, West Africa. These studies have examined whether antimalarial treatment, or episodes of parasitemia, alter antibody responses, T cell markers, and/or protective efficacy/activity following vaccination with approved routine vaccines (N=45; Euvax® or TWINRIX® and Menactra®), a whole organism malaria vaccine (N=30, PfSPZ Vaccine) and a transmission blocking vaccine (N=120; Pfs25H-EPA/Alhydrogel®). Data from all three studies will be presented examining the impact of antimalarial treatment or of incidental malaria episodes on T cell exhaustion and regulation, as well as on vaccine responses.

1608

PATTERNS OF ANTIBODY RESPONSES TO *PLASMODIUM FALCIPARUM* INVASION LIGANDS ACROSS DIFFERENT ENDEMIC POPULATIONS IN WEST AFRICA

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Plasmodium falciparum uses a large repertoire of parasite proteins for invasion of erythrocytes, which appears to serve as an immune evasion mechanism, making it difficult to identify targets of invasion inhibitory responses. It is possible that endemicity influences gene expression of invasion ligands and receptor preferences of *P. falciparum* clinical isolates. Therefore, we hypothesized that antibody responses to parasite invasion ligands in individuals living in endemic areas would differ and also correlate with parasite ligand gene expression. To examine this hypothesis, plasma samples from 528 children (2-14 yrs) with malaria across four endemic areas in Ghana (Accra, Kintampo, Navrongo and Hohoe) and one endemic site in Nioro du Sahel in Mali were tested by ELISA for antibodies to *P. falciparum* invasion ligands, including EBA 175, EBA140, EBA181, Rh2, Rh4 and Rh5. The seroprevalence of antibodies to the different antigens ranged from 8% to 70% among the clinical cases tested in this study. Consistent with previous reports, seroprevalence to all the antigens

increased in an age-dependent manner and antibody responses to all antigens were negatively correlated with parasite density. When expressed relative to total antibodies detected, anti-Rh2 levels were significantly higher while anti-Rh4 levels were lower in the Kintampo, Navrongo and Hohoe compared to Nioro du Sahel. Altogether, our data reveals patterns of antibody responses to specific invasion ligands that may be influenced by multiple host and parasite factors including parasite biology, age and endemicity, and deeper understanding of how these factors interplay may be important in identification of potential blood stage vaccine targets.

1609

ISOLATION AND CHARACTERIZATION OF HUMAN MONOCLONAL ANTIBODIES TO *PLASMODIUM VIVAX* DUFFY BINDING PROTEIN FROM MALARIA EXPOSED INDIVIDUALS FROM BRAZILIAN

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Plasmodium vivax merozoites recognize specific receptors on the host cell surface to selectively invade reticulocytes. A critical parasite ligand is the Duffy binding protein (PvDBP), expressed in micronemes, which binds specifically to an erythrocyte membrane glycoprotein known as Duffy blood group antigen/receptor for chemokines (DARC). Antibodies to the cysteine-rich domain II of PvDBP can inhibit binding of this parasite ligand to DARC and inhibit *P. vivax* invasion of reticulocytes *in vitro*. In our previous study with an Amazonians population, we found individuals with high-level BIAb responses (> 80% binding inhibition) developed in 26.6% of subjects under conditions of low malaria endemicity that prevail in Amazonia. Once acquired, high-level BIAb responses were predominantly PvDBP variant-transcending and that subjects with the strongest BIAb response had a >40% decrease in the risk of clinical vivax malaria during the follow-up, compared to those with the weakest BIAb response. We obtained PBMCs from 7 Amazonians with high levels of BIAbs, and sorted single PvDBPII-specific IgG+ memory B cells from two individuals, PCR amplified their IgG heavy and light chain variable regions, and cloned them into a human IgG expression vector to generate a panel of human monoclonal antibodies (mAbs). We found one mAb which recognized PvDBPII. We are now characterizing this mAb in terms of *P. vivax* DBPII strain-specificity, the PvDBPII epitopes it recognize, affinity for PvDBPII, and ability to block *P. vivax* invasion of reticulocytes *in vitro*.

1610

INVESTIGATING A POTENTIAL ROLE FOR TH1-POLARIZED TFH CELLS IN DRIVING ATYPICAL MEMORY B CELL EXPANSION IN MALARIA

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Malaria-specific antibody responses are short lived in children, leaving them susceptible to repeated bouts of clinical malaria. The B and T cell biology underlying short-lived antibody responses to malaria remains unclear. We recently found that chronic malaria exposure is associated with a large increase in atypical memory B cells (MBCs) that express inhibitory receptors and exhibit stunted BCR signaling and impaired B cell proliferation, cytokine production and antibody secretion. T follicular helper (Tfh) cells are known to play a critical role in helping B cells and generating long-lived antibody responses. In other recent work we demonstrated that acute febrile malaria in children preferentially activates Th1-polarized PD-1+CXCR5+CXCR3+ memory Tfh (Tfh-1) cells that exhibit impaired B cell help. In ongoing work we aim to understand the impact of malaria-induced Tfh-1 activation on the B cell response to malaria. Our preliminary findings suggest that Tfh-1 cells contribute to the expansion of T-bet+ B cells that phenotypically resemble atypical memory B cells—providing a potential link between the quality of Tfh cell responses to malaria and atypical MBC expansion.

1611

HUMAN ANTIBODIES IN MALARIA: STRUCTURE, FUNCTION, MECHANISM AND NEUTRALIZATION

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The design of effective malaria vaccines will require harnessing the human antibody response to produce broadly-neutralizing antibodies against *Plasmodium* antigens, which contain both protective and non-neutralizing epitopes. Highly-immunogenic non-neutralizing epitopes produce high-titers of non-protective antibodies and limit the production of neutralizing antibodies against protective epitopes. Therefore, accurate human B cell epitope maps of *Plasmodium* antigens are necessary to identify and retain protective epitopes, while eliminating highly-immunogenic non-protective epitopes for vaccine designs. We will present the first structural and functional data on naturally-acquired human antibodies that target *Plasmodium* antigens. Human monoclonal antibodies were isolated, cloned, expressed, and purified from individuals exposed to malaria. X-ray structures of antibodies in complex with *Plasmodium* antigens provided high-resolution definition of the epitopes and of the mechanisms of neutralization. In addition to crystallography, epitopes were identified by mutational, computational, and biophysical methods in a combinatorial approach. Within a given antigen, the most effective inhibitory human antibodies share a protective epitope, prevent the function of the antigen, and appear to be strain-transcending. Strikingly, the epitopes recognized by human monoclonal antibodies are distinct from neutralizing epitopes defined by mouse vaccinations, emphasizing the need to study human antibody responses as results derived from murine studies may not translate to human immunology and likely confound the design of human vaccines. These studies provide comprehensive explanations of human antibody neutralization mechanisms and expand our understanding of the function of *Plasmodium* antigens. These data, in combination with other data on epitopes known to be broadly-neutralizing, will improve the development of next-generation protective vaccines.

1612

ELUCIDATING NATURAL KILLER CELL-MEDIATED ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY TOWARDS RED BLOOD CELLS INFECTED BY *PLASMODIUM FALCIPARUM*

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In malaria endemic areas, humans develop clinical immunity only after years of recurrent exposure. This naturally acquired immunity depends primarily on antibodies specific for parasite antigens. The underlying basis of this protective response remains unclear. In particular, the contribution of antibody-dependent cellular cytotoxicity (ADCC) to malaria immunity remains unclear. Primary human natural killer (NK) cells from peripheral blood exhibit potent ADCC through $Fc_{\gamma}RIII$ (CD16) binding to IgG-coated target cells. Without specific antibodies, NK cell-mediated natural cytotoxicity towards both uninfected and infected RBCs was undetectable. Addition of serum from rabbits immunized with human RBC, however, resulted in NK cell-dependent lysis of both uninfected and infected

RBC. Rabbit serum antibodies specific for PfEMP1, an immunodominant *P. falciparum* variant antigen at the surface of infected RBC, induced selective lysis of infected RBC by NK cells. Plasma from malaria-immune individuals also triggered selective NK-mediated ADCC of infected RBCs. Granzyme B, a serine protease released by cytotoxic lymphocytes, is a key effector of target cell death. Granzyme B activity was detected in infected RBCs during NK-mediated ADCC using a granzyme B reporter in live cells. Furthermore, the general serine protease inhibitor DCI blocked RBC lysis. These results suggest that granzymes contribute to NK-mediated cytotoxicity towards infected RBCs. Using time-lapse imaging, dynamics of granzyme B delivery into infected RBC and subsequent outcome will be analyzed. Furthermore, NK-mediated ADCC triggered by antibodies from malaria-immune individuals inhibited *Plasmodium falciparum* growth, as determined by the re-invasion of fresh, uninfected RBC. Therefore, primary human NK cells have the potential to limit the growth of blood-stage *P. falciparum* through specific ADCC-mediated lysis of infected RBC.

1613

TRANSPLACENTAL TRANSFER OF MATERNAL MALARIA-SPECIFIC IGG3 IS ALTERED BY A POLYMORPHISM IN THE BINDING DOMAIN TO FCRN

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Transfer of protective maternal IgG to the fetus occurs by active transport via the Fc Receptor (FcRN) on the syncytiotrophoblast cells of the placenta, contributing to antibody-mediated protection against malaria in early infancy. IgG1 and IgG3 subclasses are the most efficiently transported antibodies. Although IgG1 subclasses dominate the immune response to many pathogens, robust malaria-specific IgG3 often occurs to many malaria proteins and have been strongly correlated with protection against clinical malaria as it is the case for the Merozoite Surface Protein 2. Interestingly IgG3 subclass is the only IgG subclass to contain a single amino acid polymorphism R435H, localized on the Fc fragment of the heavy chain, that affects the *in vitro* IgG3 binding of IgG3 to FcRN *in vitro*. Indeed, the R435 proteoform is associated with a reduced binding). Based on a cohort of mother-newborn pairs from a malaria endemic area of Benin (N=527), where the R435 allele frequency is 80%), we show for several asexual blood stage antigens a reduced efficiency of the transplacental transfer of malaria-specific IgG3 relative to IgG1 to the same blood stage antigen (MSP1, MSP2-3D7, MSP2-FC27, MSP3, Apical Membrane Antigen 1, Glutamate-Rich Protein region R0 and R2). This impaired transport is associated with the R435H polymorphism (p=0.01) after adjustment on for malaria exposure, and *P. falciparum* placental malaria infection. Thus transplacental transfer of malaria-specific IgG3 can be impaired by an individual polymorphism in the binding domain of the Fc fragment to the FcRN and may affect the efficacy by which of newborns acquire sufficient levels of protective antibodies to combat malaria.

1614

EVALUATING THE ANTIMALARIAL ANTIBODY RESPONSE TO SEVERE *PLASMODIUM FALCIPARUM* MALARIA IN UGANDAN CHILDREN: A CASE-CONTROL STUDY

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Severe malaria remains a leading cause of morbidity and mortality worldwide. The brunt of the disease is borne by children in the sub-Saharan region where *Plasmodium falciparum* is endemic. This sub-population is at a higher risk of developing severe disease because it is only beginning to develop immunity to the disease. Epidemiologic studies have established that individuals in endemic areas acquire immunity through repeated exposure over many years, but this is not fully protective. We conducted a prospective study in Uganda, a malaria endemic area, to evaluate the antibody responses to several well characterized sporozoite and merozoite *P. falciparum* antigens. The study enrolled 711 children and followed them for a year with data collection completed every 6 months. We compared antibody responses in 498 children diagnosed with severe malaria, in the form cerebral malaria or severe anemia due to malaria, to that in 213 healthy controls matched for age and place of residence. Preliminary data indicate that, at enrollment, children with severe malaria had significantly higher antibody levels to CSP, EBA-140, EBA-175, EBA-181, MSP-2, MSP-3 and SERA5 antigens (all $p < 0.0001$) with no diminution of statistical significance following Bonferroni adjustment for multiple comparisons. Data analysis, including evaluation of response to 5 unique PfEMP1 antigens, is ongoing.

1615

TESTING ANTIGEN INTERFERENCE ON A MULTIPLEX PLATFORM FOR MALARIA VACCINE RESEARCH

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The enzyme-linked immunosorbent assay (ELISA) is a technique, commonly used to measure antibody responses in serum or plasma samples. A traditional limitation of this technique is that, individual testing is required for every antigen against which samples are evaluated. With the introduction of multiplex detection assays, such as the one developed by the Luminex Corporation (Austin, Texas), more than one analyte can now be measured simultaneously. Such assays use fluorescent coded microspheres to which individual antigens or antibodies are covalently linked. The luminescence generated from each microsphere is used to quantify the amount of antigen/antibody present in a given test sample. This technique is currently being used for the multiplex detection of malaria antibodies in serum/plasma samples.The expanded capabilities of multiplex assays bring their own inherent challenges secondary to the potential for unintended protein- protein interactions. Such interactions may alter the measurable antigen concentrations or the antibody binding affinity, leading to antibody interference, higher background signals and decreased assay sensitivity. It is thus crucial to identify any interferences that may occur in assays, in order to provide acceptable ranges for each of the multiplexed antigens in the given test serum.We tested for the interference of different malarial antigens on a Luminex multiplex platform. Recombinant full length CSP, peptides of (NANP)6 antigen and Pf16, three antigens known to impact immunity in malaria, were used. These antigens were tested in various combinations of up to four antigens

per assay, on a standard platform with blank microspheres as controls. Data were presented in Median Fluorescent Intensity (MFI). The results will be discussed.

1616

DEFINING MOLECULAR ADJUVANT EFFECTS ON HUMAN B CELL SUBSETS

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The development of malaria blood stage antigen vaccine has been difficult and there has been only limited success to date in eliciting a potent and protective immune response in humans given these vaccines. The overall goal of this project is to identify and characterize new molecular adjuvants and adjuvant combinations that may be used in vaccines, including a malaria vaccine, to induce human B cell development and activation and to promote T follicular (Tfh) helper cell differentiation. To this end our lab examined the stimulation properties of six PRR ligands: R848 (TLR7/8), GLA (TLR4), iE-DAP (Nod1), poly(I:C) (TLR3), TDB (Mincle), and CpG (TLR9), on three human B cell subsets at different developmental stages, the immature transitional B cells, the mature marginal zone and the follicular B cells. These B cell subsets are part of normal B cell development and response to infection or vaccination via PRR ligand recognition and may affect the differentiation and activation of these B cell populations, as well as downstream Tfh cell interactions. Extensive research in mice has shown that PRR ligands affect B cell differentiation and activation, however the interaction of these molecules with these B cell populations has yet to be fully investigated in humans. Transitional B cells in human peripheral and cord blood were stimulated with various PRR ligands to determine their ability to mature transitional B cells to either a marginal zone or follicular B cell phenotype. Following stimulation, the majority of immature transitional B cells differentiated into a mature follicular B cell phenotype. Marginal zone and follicular B cells were isolated from human tonsils and stimulated to determine their ability to up-regulate activation markers like CD86. Following stimulation, tonsil-derived marginal zone and follicular B cell CD86 expression increased in response to TLR7/8 and TLR9 ligands. Understanding how PRR ligands affect human B cell subset differentiation and activation will give insight into their ability as vaccine adjuvants to drive the human adaptive immune response.

1617

IDENTIFICATION OF PROTECTIVE B-CELL EPITOPES WITHIN PFSEA-1, A NOVEL VACCINE CANDIDATE FOR *PLASMODIUM FALCIPARUM* MALARIA

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We discovered *Plasmodium falciparum* Schizont Egress Antigen-1 (PfSEA-1) by whole proteome differential screening using plasma from resistant and susceptible children living in a holoendemic region of Tanzania. Naturally occurring antibodies to the immunorelevant region of PfSEA-1 (aa 810-1023; PfSEA-1A) protect young children from severe malaria and vaccination of mice with PbSEA-1A protect against *P. berghei* ANKA challenge. To identify protective B-cell epitopes in PfSEA-1A, we vaccinated non-human primates (*Aotus sp*; n=7) with rPfSEA-1A. We performed linear, B-cell epitope mapping of PfSEA-1A using anti-sera collected from vaccinated animals and screened microarrays containing 15mer overlapping peptides spanning PfSEA-1A. These serum samples recognized 5 unique, linear B-cell epitopes within PfSEA-1A. We next determined if antibodies to these 5 epitopes were associated with protection from parasitemia following treatment in a cohort (males; age 7-30) from a holoendemic region of western Kenya. Volunteers were enrolled and drug cured of malaria infections at the start of a high transmission season, and followed with weekly blood films (18 wks) to assess reinfection. Blood was collected for serologic assays 2 weeks post treatment, prior to reinfection. We synthesized 5 peptides (~25aa) each containing one of the identified epitopes, coupled them to Luminex microspheres, and measured anti-peptide IgG antibody levels in the 2wk post treatment sera collected (n=141). When analyzed as continuous antibody levels in GEE models, IgG responses to epitopes 1, 4, and 5 predicted significantly decreased parasitemia over 18 weeks of follow-up (P=0.005-0.015). When analyzed dichotomously, individuals with high antibody levels (≥median) to these epitopes had 25-26% decreased parasitemia (P=0.009-0.012) over the 18 wks of follow-up compared to individuals with low antibody levels (<median). To advance the development of PfSEA-1 as a vaccine candidate, we are now designing immunogens targeting antibody responses to these three protective epitopes and have begun work to identify the Tfh-cell epitopes driving these responses.

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DISTINCT EXPRESSION PATTERN OF INHIBITORY MOLECULES ON CD4+ T CELLS IS ASSOCIATED WITH UNCOMPLICATED VERSUS COMPLICATED MALARIA

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Infections with *Plasmodium falciparum* (Pf) can lead to a wide clinical spectrum, ranging from life-threatening malaria to asymptomatic infections. The immune response of the infected host is one of the main factors influencing the clinical picture of a Pf infection. In endemic areas, regularly exposed children over years develop a "clinical immunity" which protects from severe malaria and is associated with mild or asymptomatic Pf infections. The immunological mechanisms involved remain poorly understood but immune tolerance has been proposed to contribute to "clinical immunity". We therefore examined the CD4+ T cell response of Ghanaian children with 1) complicated malaria, requiring inpatient treatment, 2) uncomplicated malaria, treated as outpatients; 3) asymptomatic Pf infection and 4) uninfected children. Using flow cytometric analysis, we characterized the expression of inhibitory molecules on CD4+ T cells such as CTLA4, PD1, TIM3, LAG3 and CD39, which play important roles in the T cell regulation in acute and chronic infections. Both groups of children with acute malaria showed high expression of PD1 and CTLA4. But children with uncomplicated malaria showed a significantly higher expression of inhibitory molecules such as TIM3 and CD39 compared to children with complicated malaria. In contrast, asymptomatically infected children expressed only low levels of inhibitory molecules. A stronger expression of inhibitory molecules is associated with a clinically milder course of acute malaria. The identification of an "optimal" CD4+ T cell response could contribute to the development of new treatment and vaccination strategies for complicated malaria.

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USING DEEP POPULATION SEQUENCING TO INVESTIGATE IMMUNE-BASED SELECTION ON ANTIGENIC *LOCI* IN *PLASMODIUM FALCIPARUM*

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It is commonly presumed that the human immune response interacts with *Plasmodium* antigens in an allele-specific manner. Until recently, however, we lacked the large-scale data to rigorously address these questions in naturally acquired infections. As part of the recent RTS,S/AS01 phase 3 trial, we obtained genetic data from over 5,000 infections at 11 study sites across Africa. This deep population sampling provides extremely high genetic resolution for three polymorphic *P. falciparum* proteins: CSP, SERA2, and TRAP. Previous analysis of these data compared vaccinated and control individuals to find that the RTS,S vaccine shows allele-specific efficacy at CSP. Here, we focus on the unvaccinated control individuals to investigate whether naturally acquired immunity exerts selective pressures and shapes patterns of polymorphism at these three loci. At CSP, but not SERA2, we found significant linkage disequilibrium both within and between putative T cell epitopes. Using a combination of haplotype-based analysis and in silico population genetic modeling, we showed that the linkage at CSP is most consistent with a model of long-term balancing selection. Further, the dataset's unprecedented size provides the power to investigate intrahost selection at all three proteins. We examined polymorphism within each infection and found regions of reduced intrahost diversity in older children compared to younger children in both CSP and SERA2. Analysis of TRAP is ongoing. This pattern is consistent with a model of immune-mediated, allele-specific selection. Combining the signals of population-level and intra-host selection, we pinpointed specific amino acids that appear to drive the evolutionary dynamics at each protein, furthering our understanding of how the human immune system may interact with, and shape the diversity of, the parasite.

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ANTIGENICITY AND TRANSMISSION-BLOCKING EFFICACY OF *PLASMODIUM VIVAX* PVS48/45 PROTEIN

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Plasmodium P48/45 are gametocyte antigens involved in parasite fertilization which induce immune responses that lead to blockage of parasite transmission to mosquito and are therefore considered candidates to develop a malaria transmission-blocking (TB) vaccines. In the process of developing *P. vivax* 48/45 as potential vaccine we recently expressed as a full length a recombinant product (rPvs48/45) and 5 internal fragments covering the entire protein. Immunogenicity studies in mice and Aotus monkeys indicate that anti-Pvs48/45 antibodies prevent the formation of oocyst in the mosquito midgut. Antigenicity studies were carried out in 235 plasma of individuals from malaria endemic areas of Colombia. Samples were assessed using the Pvs48/45 full-length protein and reactivity in 160 samples using five sub-fragments recombinant products by ELISA. Overall response indicated that 75.3% (177/235) of the sera recognized the full-length protein, whereas the N-term fragment encompassing the sequence between a.a.14 and a.a.186 was the most frequently recognized, 35.6% (57/160). Although all other fragments were recognized, their reactivity ranged between 15.5 - 21.7%. Transmission blocking assays in 152 samples tested showed that ~10% of these sera contains specific *P. vivax* antibodies with high TB activity (90-100 %), 51% showed an intermediate (50-90%) TB activity and 38% presented low (0-50%) TB activity. Furthermore, affinity purified anti-Pvs48/45 cross-reacted with *P. falciparum* gametocytes by IFAT. These results confirm the high antigenicity of Pvs48/45 and identify the N-term fragment as the most antigenic. The functional activity of affinity purified specific anti-Pvs48/46 N-term fragment is being tested.

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HUMAN CORD BLOOD CXCR5+ CD4 T CELLS: ASSOCIATION WITH *IN UTERO* EXPOSURE AND ANTIBODY RESPONSES TO *PLASMODIUM FALCIPARUM*

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Fetuses exposed to *Plasmodium falciparum* (Pf) from infected mothers can make anti-malarial antibodies but it is unclear if long-lived plasma cells (LLPC) and memory B cells (mBC) contribute to the antibody production *in utero*. T-follicular helper cells (TFH) in adult germinal centers provide help for primed B cells to differentiate into LLPC and mBC and CXCR5-expressing CD4 T cells in peripheral blood are circulating counterparts of TFH. The present study investigated whether CXCR5+ CD4 T cells are present in umbilical cord blood and if they are associated with *in utero* exposure and antibody responses to Pf antigens. Expression of CXCR5 messenger RNA (mRNA) was quantified by real-time PCR in CD4+ T cells isolated from peripheral blood mononuclear cells (PBMC) of 30 Cameroonian neonates and 6 Cameroonian adult controls. Day 5 supernatants of neonatal PBMC cultures were tested for IgM and IgG to a panel of blood-stage Pf antigens using the MagPix. Placental malaria (PM) was assessed by microscopic examination of placental impression smears. A total of 20 out of 30 neonates (66.7%) had detectable CXCR5 mRNA with 20% having higher CXCR5 expression than the least expressing adult sample and 3.3% having expression levels comparable to the median adult expression. The presence of PM significantly increased CXCR5 expression ($p=0.016$) and in 55% of neonates, *in vitro* treatment of PBMC with MSP-1 antigen also increased CXCR5 expression. Also, 0% and 16.7% of culture supernatants tested positive for Pf IgM and IgG respectively but CXCR5 expression levels did not correlate with IgG levels. Collectively, the data show that circulating TFH-like cells can be produced *in utero* and their frequency increases in response to fetal Pf exposure. Supplemental studies are on thee way to determine if Pf-specific mBC and LLPC are generated *in utero*.

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PLASMODIUM FALCIPARUM WHOLE PROTEOME ANTIBODY PROFILES OF EUROPEAN VOLUNTEERS IMMUNIZED WITH SPOROZOITES UNDER CHLOROQUINE CHEMOPROPHYLAXIS

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Malaria remains a major disease burden in developing countries, killing over 438,000 people in 2015. Controlled human malaria infection (CHMI) has allowed for rational vaccine discovery and development.

Immunizations with both sporozoites from mosquito bites (CPS) and live, metabolically active cryopreserved sporozoites (CVac) under chloroquine Chemoprophylaxis in naïve healthy individuals induces dose-dependent sterile protection against sporozoites, but no immunity against blood stage infection. Understanding the antibody immune response in this immunization strategy is an important step in elucidating the mechanism of protection and designing next generation vaccine strategies. To understand antibody responses associated with protection, whole proteome microarrays covering 91% of the *P. falciparum* proteome were developed. We probed and analyzed serum samples collected from 39 Dutch individuals in three clinical trials who had undergone CPS immunization and 27 German volunteers in a trial of CVac at time points before and after immunization and CHMI. Protected CPS-immunized individuals showed a dichotomous antibody profile: low and high responders. High dose recipients had a broader repertoire of antibody reactivity compared to medium and lower dose recipients. Unprotected individuals showed boosting of antibody levels after CHMI. Only antibodies against CSP were boosted in protected individuals. In CVac recipients, liver stage proteins had the highest seropositive rate. The high dose group (9/9 protected) had a larger network or immunoreactive proteins than the low and medium dose groups (3/9 and 6/9 protected, respectively) in bipartite network analysis. Dichotomous antibody profiles suggest two mechanisms of protection: 1) early protection that prevents increased parasitemia and limits antigen exposure, and 2) delayed protection at the late liver stage with greater antigen exposure. Correlates of susceptibility likely illustrate higher levels of antigen exposure due to more frequent and greater blood stage parasitemia.

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RAPID ASSESSMENT OF A NATIONWIDE LONG LASTING INSECTICIDAL (MOSQUITO) NETS DISTRIBUTION CAMPAIGN IN BENIN

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In 2014 Benin's National Malaria Control Program (NMCP) distributed more than 6 million long-lasting insecticidal nets (LLINs) through a national mass distribution campaign. The NMCP assessed post-campaign household (HH) coverage to help inform decisions about future campaigns and distributions. Random cluster sampling of villages and city neighborhoods was conducted in Benin's 12 geographic departments. The final sample included all of Benin's 34 health zones. Each health zone contained 30 clusters containing 10 HH each. Data were collected through interviews and direct observation of LLIN ownership and use. Population based estimates of universal coverage rate (one net per two persons), LLIN use and respondents' knowledge of malaria were generated. Of 10,002 HHs surveyed, 88% (95% CI: 87.2, 88.5) received a campaign coupon to claim a free net. Of these HHs, 89,857 (89%) provided information on the number of LLIN received and 8,528 (85.%, (95% CI: 84.5, 85.9) reported receiving at least one LLIN. Less than 3% of HHs received a coupon, but did not receive at least one LLIN. Fifty-six percent (95% CI: 55.4, 57.5) of HH receiving an LLIN during the campaign received 2-3 LLINs. Approximately 76% (95% CI: 75.3, 77.2) of HHs receiving LLIN during the campaign reported adequate coverage (at least one LLIN for two persons). The gap in "adequate" coverage during the mass distribution campaign was in part due to an underestimate of the population in need of LLINs and a gap in the number of LLINs available for distribution during the campaign. The survey also found that 77.2% (95% CI: 76.4, 78.0) of HHs used an LLIN the previous night, and 89% (95% CI: 88.5, 89.7) of HHs demonstrated a good knowledge of the benefits of LLIN use for malaria prevention. Although this initial post-campaign assessment found that a high proportion of HHs received at least one net and the majority of HHs received an adequate number of nets during the national campaign, additional investments in campaign planning and logistics are needed to improve national universal coverage in Benin.

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DATA QUALITY ASSURANCE AND DATA MANAGEMENT IN A LARGE SYSTEMS BIOLOGY PROJECT: MAHPIC

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Large systems biology projects, such as the NIH/NIAID supported Malaria Host Pathogen Interaction Center - MaHPIC involve several collaborating research centers. Often, each center is specialized in different aspects of the system and each generates different data types such as genomics, transcriptomics, proteomics, metabolomics, lipidomics, immune profiling, clinical data, interaction models etc. Often, each center is accustomed to their own protocols, standards, and research practices, as dictated by their respective fields of specialization. However, together, they generate thousands of files that occupy terabytes of storage space. These data include raw and intermediate data (processed/normalized), result files, metadata, SOPs and other supporting documents. Data integration and mathematical modeling are the cornerstones and preferred approaches for systems biology research. However, for data integration and modeling to be possible, high-quality data in defined formats are necessary to make them computationally tractable. In the face of the high volume, high variety and high velocity of data generation, ensuring quality, accuracy, and accessibility to modelers, the team and the research community is a huge challenge. Solutions require rigorous standards, and willing participation by all involved. The Informatics Core of the MaHPIC works closely with all data producers and consumers to implement standards (when they exist) and to develop and/or implement rigorous protocols for data collection, validation, transformation, and dissemination. The solutions we have developed include metadata and result templates designed for each data type, data transfer protocols that include a pre-transfer review of data, validation scripts and procedures, a dedicated file repository, a relational database for rapid data access by mathematical modelers, ontological mark-up of experimental processes and results, and several Web-based resources managed under a single project Portal. This high level of quality control for rich, well-curated data creates a valuable resource that should permit discoveries for years to come.

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FLUORESCENT LABELLING OF WILD TYPE *PLASMODIUM* SPECIES WITHIN THE MOSQUITO HOST: A NOVEL METHOD TO TARGET SPOROZOITES

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Mutant *Plasmodium* parasites expressing fluorescent proteins allow preclinical imaging of malaria development and distribution in both cell lines and animal models. However, the widespread application of genetically altered organisms is limited, due to regulatory constraints and the inability to culture some *Plasmodium* species, such as *P. vivax*. As such, reporter lines are not available for all *Plasmodium* species. This calls for more generic approaches that allow for the targeting and (molecular) imaging of sporozoites. Here we present a novel method to fluorescently label the sporozoite stage of wild-type *Plasmodium* species *in vivo* and without the need for genetic modification or the extraction of the parasite from its mosquito host. *In vitro* studies demonstrated a tailored fluorescent

cyanine-5 (Cy5) dye could efficiently stain sporozoites *in vitro*. By membrane-feeding infected *Anopheles* mosquitoes on glucose using the exact same dye we were even able to specifically label sporozoites within the mosquito's salivary glands *in vivo*. The Cy5-dye was preferentially taken up by the mitochondrion of sporozoites and the uptake therein was higher compared to native mosquito tissue such as salivary gland cells or cells of the midgut. This specificity indicates that the mitochondrial activity of sporozoites provides a valuable (*in vivo*) targeting mechanism. To demonstrate cross-species utility of this technology, it was successfully applied in *Plasmodium yoelii*, *berghei* as well as *falciparum*. Viability of the fluorescently labelled sporozoites was confirmed in a hepatocyte cell line. Targeting plasmodium sporozoites through the feed removes the need for genetic modification with imaging vectors, thereby allowing more detailed studies for species such as *Plasmodium vivax*. In addition, the specificity of the mitochondrial uptake that we observed, suggests this to be a possible molecular targeting route for sporozoites residing in the mosquito host.

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THE IMPACT OF REVISED HEALTH MANAGEMENT INFORMATION SYSTEM (HMIS) REPORTING FORMS ON THE QUALITY OF MALARIA SURVEILLANCE DATA IN UGANDA: AN INTERRUPTED TIME SERIES ANALYSIS

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Malaria control programs need accurate data to implement and evaluate malaria interventions. In July 2015, Uganda introduced revised HMIS reporting forms to health facilities (HFs) to improve data quality. To evaluate this intervention, we assessed data completeness and accuracy in five HFs in Kayunga district. We abstracted data from 7,523 records in outpatient (OPD) registers and surveillance summary reports for 12 months before and four months after the intervention. Monthly completeness was measured as the proportion of malaria patient records with: 1) all data fields completed, and 2) clinically-relevant fields completed. Accuracy was the relative difference between numbers reported in the OPD register and surveillance reports for total patients, malaria patients, malaria tests performed, and positive malaria tests. Data were analyzed as interrupted time series with segmented linear regression. The current analysis is limited to one HF with complete time series available; data collection for other HFs is ongoing. Completeness for all data fields ranged from 0-14% over time, with no effect of the intervention (*P*-value of instantaneous change [*P*(i)]=.94, *P*-value of slope change [*P*(s)]=.66). Completeness of clinically-relevant fields, which averaged 30% at baseline, showed an improvement of 38 percentage-points immediately following the intervention (95% CI: 0.28-0.49; *P*(i)<.0001). This increase was driven by improvement in recording patients' weight. Discrepancies between surveillance reports and registers ranged from 0-15% for all patients, 1-9% for malaria patients, 24-71% for tests performed, and 0-20% for positive tests, with no significant intervention effect. In conclusion, revised reporting forms improved completeness for clinically-relevant data but had no effect on data accuracy. Analysis of additional HFs will assess intervention effectiveness in a broader setting.

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IMPACT OF THE APPLICATION OF THE NEW GUIDELINES OF MALARIA CASE MANAGEMENT IN SENEGAL

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Since 2013 The NMCP has made a review of its management policy and malaria prevention with the introduction of the new guidelines as recommended by the WHO. . So in February 2014, we updated the training book, the facilitator and participant's guide with the support of all stakeholders . With the mobilization of 15 trainers from the central level, between February and April 2014, the agents of medical regions were oriented and 312 out of 328 were trained that makes a total participation rate of 95% with a sex ratio of 54% of men and 46% women. At the operational level, among the 2991 agents which were provided for, 2951 were trained that is to say a participation rate of 98% covering all the districts in the country with a sex ratio of 42% women and 58% men. At the community level, 50 community health workers were trained with 36 from health huts and 16 from ICCM sites with a focus on pre-referral treatment of severe cases. Thus between 2013 and 2015, there has been a positive improvement of some indicators of malaria case management in health structures resulting in a remarkable rise of the screening rate from 87.49% to 99.3%, the rate of ACT distribution from 98.3% to 99.5%, the rate of the 2nd of IPTp coverage from 65.93% to 70.18% with an effective implementation of the 3rd dose of IPTp amounting to 42.72% in 2015. In the same year, 7684 pregnant women with simple cases of malaria were effectively managed with ACT according to the new guidelines. . The management of severe cases by the injectable Artesunate became effective in 2015 at 07 pilot health units with a cure rate of 90% among the enrolled cases. All these positive results have shown that the implementation of the new guidelines can considerably improve the management of malaria at the level of prestation places and reduce the impact of morbidity and mortality at the same time. The NMCP is prospecting to continue the training of new providers but also to ensure a steady supervision of the trained staff to maintain the achievements in terms of capacity building in order to fully pre-eliminate malaria in Senegal.

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THE "DYNAMIC EPIDEMIOLOGY" OF MALARIA ELIMINATION IN EL SALVADOR: THE ROLE OF PROGRAM DECENTRALIZATION, STRATIFICATION, AND TIMELY TREATMENT IN THE RAPID AND DURABLE DECLINE IN MALARIA INCIDENCE SINCE THE EARLY 1980S

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Resurgence of malaria cases in the 1970s following the end of the Global Malaria Eradication Program led El Salvador to re-evaluate and alter its national malaria control strategy. By the early 1980s, El Salvador had the highest burden of malaria in Mesoamerica (95,835 cases in 1980, ~20% being *Plasmodium falciparum*). In 1995 El Salvador had its last autochthonous *P. falciparum* case. Today, it is on the verge of malaria elimination with fewer than 20 *P. vivax* cases per year since 2011 while its immediate neighbors continue to have the highest malaria incidences in the region. We reviewed and evaluated the policies and interventions implemented by the Salvadoran national malaria control program that likely contributed to this progress toward malaria elimination. Decentralization of the program, early regional stratification by risk, and

stratum-specific program actions resulted in the timely and targeted allocation of resources toward vector control, surveillance, and infection detection and treatment. The presumptive treatment regimen of combined chloroquine + primaquine was also shortened to five days, which greatly improved compliance. Importantly, weekly reporting by health workers and volunteer collaborators distributed throughout the country by strata, and informed via a reliable digital information system, enabled local malaria teams to provide rapid, adaptive, data-based responses in a locally focused manner leading to the description of the program in El Salvador in the 1980s and 1990s as "dynamic epidemiology". Data-based adaptation of the program continues to yield favorable results to maintain pre-elimination levels, with most of the current cases being imported from neighboring countries. Additional support for systematic elimination efforts in neighboring countries, potentially learning and adapting from the El Salvador experience, will undoubtedly benefit each of these countries and may be required for El Salvador to fully achieve malaria elimination. El Salvador provides a relevant country case study and learnings can guide application of similar strategies in other countries approaching malaria elimination.

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MOVING TOWARD IMPROVED MEASUREMENT OF MALARIA MORTALITY AT THE POPULATION LEVEL

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Measuring malaria-specific mortality at the population level is challenging due to the difficulty in assessing malaria and the fact that most malaria deaths occur outside of the formal health system. To address this gap, the verbal autopsy (VA) method was developed to ascertain cause of death at the population level, yet there are limitations with current VA tools and approaches for measuring malaria-specific mortality. Given the emphasis in the new Global Technical Strategy for Malaria on monitoring malaria-specific mortality, there is a strong need for the malaria community to develop improved methods for measuring malaria-specific mortality. To help inform a strategy for improving current methods, we carried out a systematic literature review to assess how VA tools and approaches have been used to measure malaria-specific mortality and the key challenges and limitations of existing tools and methods. A key limitation we found was the varying and overall low levels of sensitivity and specificity of VA tools for measuring malaria mortality, due to the non-specific symptoms of malaria and the differing malaria epidemiological contexts in which studies are conducted which result in misclassification bias (either over- or under-estimating the burden). Further, most VA validation studies use hospital records as the gold standard to compare VA results, yet these are not a true gold standard since it is a reflection of a different population and are often incomplete. Comparability of malaria mortality results across study sites was also a challenge, due to a lack of standardization in the application of VA tools and methods and the limited details provided overall in many published VA studies on the methods and tool used, including how malaria cause of death is determined. Given these limitations, we propose using community estimates of mortality measured through VA and complementing them with in-patient mortality data from health facilities that incorporate malaria parasitological confirmation to produce more robust population-level estimates of malaria mortality.

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MEASURING THE IMPACT OF MALARIA ON HEALTH-RELATED QUALITY OF LIFE OF CHILDREN IN RURAL WESTERN KENYA

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The aim of our study was to assess the impact of malaria on health-related quality of life (HRQoL) using subjective patient reported measures. We used purposive sampling at outpatient and inpatient hospital wards to select our sample from those families seeking care at a private facility in rural western Kenya, an area with high baseline malaria parasitaemia. Our subjects met the following criteria: i. Child under age 15 years; ii. Laboratory-confirmed malaria. We collected information from parents or caregivers of 64 children, age 4 months - 13 years via audio recorded interviews at the time of receiving medical care and then in person or by phone 5-7 days after the health facility visit. Using mixed methods, we gathered narrative and survey information on the child's baseline health prior to the current illness and data on quality of life from the time of illness onset through recovery, such as the ability to play, go to school, or participate in normal activities. Overall, 42% of our sample was hospitalized children. Mean HRQoL values were 0.7312 SE 0.0161. HRQoL values were inversely correlated with high fever (pwcorr -0.8830, $p < 0.001$; Bonferroni adjustment), with the lowest rating of HRQoL occurring between 1 and 4.5 days after the onset of symptoms. We converted HRQoL to a summary outcome measure, Quality Adjusted Life Years (QALYs). The mean QALYs lost per child during a treated episode of malaria in our sample were 0.005 (SE 0.0006, 95% CI 0.0044-0.0056). Generalizability of our results was limited due to the high proportion of hospitalized children in our sample, reflecting greater severity of illness than would be experienced by children who were not hospitalized. Nevertheless, our study offers the first time that HRQOL values have been mapped, and QALYs calculated for pediatric malaria.

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THE CATASTROPHIC IMPACT OF MALARIA ON HOUSEHOLDS IN RURAL WESTERN KENYA

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We measured the impact of pediatric malaria on the total value of out of pocket payments and opportunity costs to households in rural western Kenya. We used purposive sampling at outpatient and inpatient hospital wards to select our sample from those families seeking care at a private facility in rural western Kenya, an area with high baseline malaria parasitaemia. Our subjects were children less than 15 years of age with laboratory-confirmed malaria. We collected information from parents or caregivers of 64 children, age 4 months - 13 years via audio recorded interviews at the time of receiving medical care and then in person or by phone 5-7 days after the health facility visit. We gathered information on illness-associated costs from travel, treatments, medical care, food, lost time from work, school, and the status of children left at home while a parent or caregiver was attending to the ill child. Overall, 42% of the sample was hospitalized children. A total of 11% of all families used herbs prior to bringing the child to the hospital, paying a

median cost of 250 Ksh, over 83% of the cost of a full pediatric course of artemether/lumefantrine. Use of herbs was significantly associated with a longer interval between symptom onset and presenting for diagnosis and treatment (Pearson $\chi 2$ 136.5, $P < 0.000$) but a lower chance of hospitalization. Even when there were no charges for direct medical care, study families incurred high costs due to lost work time and out of pocket costs for transport. We found that total costs for a single treated episode of pediatric malaria in our sample represented Ksh 1750 or USD 20.59, which is 34% (sd 0.24) of the estimated median monthly household consumption of Ksh 5000 or USD 58.82 in rural Kenya. We found that significant components of costs to households during an episode of malaria were pre-hospital payment for medicinal herbs, and the gap in time from the first symptoms of illness until arrival at the health facility. In many cases, pre and post treatment costs alone imposed a catastrophic burden on families, forcing parents to sell assets, borrow money, or reallocate existing funds for school fees to cover the costs of care.

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IMPROVING PRIVATE SECTOR MALARIA CASE SURVEILLANCE AND QUALITY ASSURANCE USING DHIS2: LESSONS LEARNED

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Ensuring private providers report on all malaria cases accurately is critical to transform malaria surveillance into a core intervention as recommended in WHO's Global Technical Strategy for Malaria (2016-2030). Equally important is ensuring the quality of fever case management (FCM) by private sector providers. However, in many malaria endemic countries, health management information systems (HMIS) for the private sector are parallel to the government or non-existent, and do not include quality of care data. In response, PSI in collaboration with partners and funded by UNITAID, developed a routine monitoring system for private providers, combining case surveillance data (provider reported) with quality of care data (supervisor reported). Data was managed through DHIS2 to allow for integration with national HMIS. Piloted in Kenya, Tanzania, and Madagascar from 2013-2016, this approach has resulted in valuable lessons learned: Provider reporting rates varied across provider type and country, and were difficult to track due to limitations in managing a dynamic network of outlets in DHIS2. Quality of care data was successfully integrated into DHIS2 and managed alongside case surveillance data to prioritize scheduling of follow-up supervision visits. Quality of surveillance data, measured through Data Quality Audits highlighted challenges in the reliability of aggregated case surveillance data; while the quality of supervision data could not be assessed as there is no universally accepted gold-standard checklist. These lessons highlight actionable conclusions. 1) Routine monitoring systems and analytics tools (such as DHIS2) need to be as dynamic as the network of providers being monitored; 2) a globally validated tool to measure quality of FCM will allow countries to validate country-specific tools; and 3) provider level reporting should be simplified and streamlined, while considering provider incentives to improve data quality. The implementation of these lessons will help strengthen malaria surveillance and quality assurance in the private health sector and therefore accelerate progress towards malaria elimination.

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METHOD FOR THE SIMULTANEOUS MEASURE OF THE LEVEL OF NINE ANTIMALARIAL DRUGS IN DRIED BLOOD SPOT SAMPLES USING LC-TANDEM MASS SPECTROMETRY AND RELATIONSHIP OF LUMEFANTRINE CONCENTRATIONS IN DRIED BLOOD SPOT SAMPLES AND IN PLASMA

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Storage and transportation of blood samples are common problems for studies in areas with a high prevalence of malaria. The dried blood spot (DBS) sampling technique is promising for that use, enabling easier storage and transportation requirements. We present a method for the analysis of antimalarials in DBS. We also show the relationship between the concentrations of lumefantrine in DBS and with the usual method of plasma sampling. We added known concentrations of amodiaquine, desethylamodiaquine, quinine, chloroquine, mefloquine, sulfadoxine, pyrimethamine, lumefantrine and desbutyl-lumefantrine in whole human blood. A 10µl aliquot of this blood was applied on a filter paper card and allowed to dry for three hours at room temperature. We took a 3 mm punch out of each dried blood spot and extracted it with 100 µl of methanol containing the stable isotopically labeled Internal Standards for all the antimalarials. We used a multiplex chromatography coupled to tandem mass spectrometry (LC-MS/MS) method for the simultaneous measure of the 9 antimalarials. We measured the concentrations of lumefantrine both in DBS and in plasma obtained in 16 healthy volunteers after they had received a single dose of artemether-lumefantrine. Lower limits of quantification were 2 ng/ml for pyrimethamine, 6 ng/ml for desthylamodiaquine, and 20 ng/ml for the other antimalarials. The inter-day variation coefficient was 2.1-15.2%. Lumefantrine concentrations measured in plasma were twice as high as those measured in DBS and were highly correlated (r=0.99). Our technique enables both precise and sensitive measurement of antimalarials in DBS, despite the low volume of blood sampled. The correlation between lumefantrine concentrations in DBS and in plasma is almost perfect. This relationship could thus contribute to defining the therapeutic ranges of lumefantrine concentrations measured in DBS. The ratio between the concentration in DBS and in plasma could reflect the distribution of lumefantrine in the different blood compartments. The DBS sampling method is suitable for antimalarials level measurements and could be convenient for epidemiological studies.

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MALARIA AND HELMINTH COINFECTION-INDUCED OXIDATIVE STRESS AND CHANGES IN ANTIOXIDANT STATUS AMONG AFEBRILE SCHOOL CHILDREN IN IBADAN, SOUTHWEST NIGERIA

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Malaria and helminth infections are common tropical diseases in Sub-Saharan Africa. Little is known about the effect of co-infection of the

two diseases on the antioxidant defense system. This study determined the effect of malaria and helminth co-infection on antioxidant status in afebrile school children. A total of 99 afebrile school children were chosen comprising 25 with helminth infection, 25 with malaria-helminth co-infection and 24 negative for both malaria and helminth infections. Malaria parasite was determined by microscopy while helminth infection was confirmed by Kato-Katz method. Plasma hydrogen peroxide (H_2O_2), malondialdehyde (MDA), protein carbonyl (PC), xanthine oxidase (XO), NADPH oxidase (NOX), myeloperoxidase (MPX), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidise (GPX), glutathione S-transferase (GST), ascorbic acid (AA) and α-tocopherol (TOC) were determined. Plasma levels of H_2O_2, MDA, PC as well as activities of XO, NOX and MPX were significantly higher in children with co-infection of malaria and helminth followed by helminth only and malaria only relative to uninfected children ($p<0.05$). GST activity, GSH and AA levels were significantly reduced while SOD and GPX activities were significantly higher in co-infected children followed by malaria only and helminth only relative to uninfected children ($p<0.05$). CAT activity was significantly higher in malaria only followed by co-infection and helminth only infected children relative to uninfected children ($p<0.05$). TOC level was significantly lower in helminth only followed by co-infection and malaria only relative to uninfected children ($p<0.05$). Malaria and helminth co-infection in afebrile school children caused a reduction in plasma antioxidant status as evident from significant increases in oxidative stress markers (H_2O_2, MDA, PC levels and activities of XO, NOX and MPX) and consequent depletion of the thiol GSH, AA, TOC and GST activity.

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HIGH THROUGHPUT IDENTIFICATION OF *ANOPHELES GAMBIAE* MIDGUT GENES INVOLVED THE INVASION OF *PLASMODIUM FALCIPARUM*

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An anopheline mosquito midgut is an important organ for malaria transmission. However, the interaction between a mosquito midgut and *Plasmodium* parasites is not well elucidated. This study aims to investigate the molecular mechanisms of *Plasmodium* invasion in midguts. First, we developed a computational algorithm to predict candidate mosquito midgut proteins based on sequences and oligo-array data. Ninety-four candidate genes were predicted in *Anopheles gambiae* mosquito midguts and expect to involve in A. gambiae and *Plasmodium falciparum* interaction. More than 90% of these candidates are novel. Next, we cloned these genes and successfully expressed 68 in insect cells. ELISA binding assay revealed that 28 recombinant proteins bound to *P. falciparum*-infected cells. Furthermore, we determined functional effects of 28 candidate genes on *P. falciparum* infection in mosquito midguts using dsRNA-mediated gene expression silencing assays. The results indicated that three genes facilitated the infection of *P. falciparum* parasites and five genes inhibited the infection of *P. falciparum* parasites in mosquito midguts. Together, these results support our hypothesis that mosquito midgut proteins play critical roles in regulating *P. falciparum* parasite transmission. Notably, the results from this project lay a solid foundation to develop novel approaches to block malaria transmission.

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RELATIONSHIPS BETWEEN TRAVEL AND RTS,S MALARIA VACCINE EFFICACY IN LILONGWE, MALAWI

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The RTS,S/AS01 vaccine was recently approved by the European Medicines Agency Phase III after clinical trials showed moderate levels of efficacy that varied between the 11 clinical trial sites throughout Africa. This study assesses the relationship between travel and vaccine efficacy in a seasonal-transmission region of sub-Saharan Africa in one of the trial sites, Lilongwe, Malawi. Travel and mobility have been shown to be significant risk factors for malaria incidence but the relationship between travel and malaria vaccine efficacy has not been studied. We followed children (5-17 months of age) and infants (6-12 weeks of age) who had been randomly assigned to either a vaccine group, vaccine with booster group, or control group. Primary efficacy was defined as development of clinical malaria (fever ≥37.5°C and *Plasmodium falciparum* parasitemia >5,000 per microliter). A travel history was collected for the 1552 trial participants at 6-month intervals throughout the 3-year study period, as well as the spatial location of each participant's household and destination of travel. During the study, 30.34% of participants who received the placebo and 32.20% of participants who received the vaccine with or without the booster traveled outside the study catchment area at least once, with travel defined as at least one night spent outside Lilongwe. Overall vaccine efficacy was 34.5% among participants who traveled and 20.0% among those who did not. Travel was significantly associated with increased vaccine efficacy when controlling for socioeconomic status, participant age, seasonality of travel, and destination of travel ($p<0.001$). The reason the efficacy is higher for participants who travel is not well understood and further study is necessary. One potential explanation is that *Plasmodium falciparum* strains outside of Lilongwe are more sensitive to the RTS,S vaccine.

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ASSESSMENT OF A TRANSGENIC *PLASMODIUM BERGHEI* PARASITE EXPRESSING *P. FALCIPARUM* CELL-TRAVERSAL PROTEIN FOR OOKINETES AND SPOROZOITES (PFCELTOS) FOR USE AS A HOMOLOGOUS RODENT CHALLENGE MODEL TO TEST VACCINE EFFICACY

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The use of animal models to simulate human malaria infection is critical for malaria vaccine development and down-selection. While nonhuman primate models may approximate clinical immunology, they do not allow testing of protection against *Plasmodium falciparum* infection. Thus, the majority of malaria vaccine development utilizes rodent models to assess immunogenicity and vaccine efficacy. Studies have shown that the Cell-Traversal protein for Ookinetes and Sporozoites (CelTOS), highly conserved among Plasmodium species, plays a major role in parasite invasion of both mosquitos and vertebrates. A unique feature of CelTOS is that cross-species protection is achievable, as evidenced by our ability to attain 60% heterologous protection against *P. berghei* malaria following vaccination with *P. falciparum* CelTOS (PfCelTOS)/ISA 720. Although protection is routinely observed with the wild type *P. berghei* challenge model, a heterologous challenge for PfCelTOS-specific responses is needed for assessing homologous responses and establishing immune correlates of protection. To address the homologous challenge issue, a

chimeric *P. berghei* parasite, where the gene encoding PbCelTOS has been replaced with coding sequence for PfCelTOS (PbPfCelTOS(r)PbCelTOS) was developed as a readout and preclinical analysis method for homologous protection in mice. Experimental data will be presented on the molecular and cellular characterization of the PbPfCelTOS(r)PbCelTOS parasite with respect to: mosquito- and mouse-stage development, antibody recognition of PfCelTOS on sporozoites using anti-PfCelTOS (monoclonal and polyclonal) antibodies , *in vivo* infectivity using our standard immunogenicity testing regimens and use as a murine parasite challenge model.

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LACK OF GEOGRAPHIC SIGNAL IN THE PATTERN OF ALLELE AND EPITOPE FREQUENCIES IN FOUR MALARIA LIVER STAGE CANDIDATE VACCINE ANTIGENS

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Both blood stage and pre-erythrocytic malaria vaccines, including the most advanced malaria vaccine, RTS,S, have shown moderate and strain-specific efficacy, highlighting the need for new vaccines with potent cross-protective efficacy. The selection of antigen variants to include in future vaccines, and in particular their constituent epitopes, will be crucial to determine the extent of cross-protection as well as regional differences in efficacy. We assessed the potential of *Plasmodium falciparum* circumsporozoite surface protein (CSP), liver stage antigens 1 and 3 (LSA1 and LSA3), and sporozoite asparagine rich antigen 1 (SAP1), as whole antigens or deconstructed into B- or T-cell epitopes, to elicit strain-transcending immune responses. To evaluate the vaccine potential of these antigens, we determined allele and epitope frequencies among 30 isolates collected from Mali, Malawi, Myanmar, and Cambodia, and the pattern of geographic distribution of those variants. DNA was isolated from leukocyte-depleted blood samples, and used to generate de novo genome assemblies using Pacific Biosciences (PacBio), and Illumina HiSeq sequencing data. Custom scripts were used to identify, extract and align the genomic sequences for each of the target loci. For CSP, only two variants were identified in the N-terminal region of the gene, with prevalences 64% and 36%; in contrast, in the C-terminal region, 17 variants were found in the T-helper cell epitope 2 (Th2R) (range: 2.6% to 21%), and eight variants in the Th3R region (range: 2.6% to 34.2%). The alleles contained between 31 and 37 NANP repeats, and three or four NVDP repeats. The distribution of the haplotypes was not determined by geography. Among these 30 isolates, we identified eight conserved B-cell epitopes and 11 potential CD4+/CD8+ CSP epitopes restricted by the most frequent HLA allele in Mali. Identical analyses were conducted for sap1, lsa1, and lsa3 genes. The results from this study suggest that conserved epitopes of CSP and LSA3 present in both West and East Africa, as well as in South East Asia, may be promising candidates for inclusion in a multi-epitope malaria vaccine.

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A CONSENSUS PROTEOME OF *PLASMODIUM VIVAX* SPOROZOITES

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Plasmodium vivax represents the most geographically distributed species of human malaria. Basic research studying *Plasmodium vivax* infectious stages has lagged behind *P. falciparum* for two principal reasons: 1. *P. vivax* infections cause morbidity, but low mortality compared to *P. falciparum* and 2. we currently lack a culture system capable of growing *P. vivax in vitro*. As a result, several research groups have characterized the proteins of the *P. falciparum* sporozoite and merozoite infectious stages but the *P. vivax* sporozoite proteome has not yet been published. A detailed understanding of the *P. vivax* proteome is critical for drug and vaccine development efforts against genetically distinct species of malaria. We performed MudPIT mass spectrometry of tryptic peptides from *P. vivax* sporozoites dissected from *Anopheles dirus* mosquitoes. *P. vivax* peptides were distinguished from Anopheline peptides by searching tandem mass spectra from tryptic sporozoite peptides against a concatenated *P. vivax* - Anopheline protein database. We identified 421 core sporozoite proteins, many of which are abundant proteins with unknown functions. Additionally we identified orthologs of previously implicated vaccine candidates including CSP and TRAP (expressed abundantly on the sporozoite surface), a putative heat shock protein, SPECT1, GAMA and GEST.

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PLASMODIUM FALCIPARUM CIRCUMSPOROZOITE PROTEIN ADJUVANTED WITH LIPOSOMAL ADJUVANT INDUCES HIGHLY PROTECTIVE RESPONSES IN C57BL/6 MICE AGAINST TRANSGENIC PARASITE CHALLENGE

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Falciparum malaria continues to be a highly lethal infectious disease among children in tropical areas of the world. The most successful malaria vaccine candidate is RTS,S (GlaxoSmithKline), which targets the circumsporozoite protein (CSP) expressed by *Plasmodium falciparum* sporozoites. RTS,S contains the central repeat region and the C-terminal region of CSP expressed on a hepatitis B surface antigen particle, but it lacks the N-terminal region. Several lines of evidence suggest the N-terminal region of CSP contains residues that are critical for hepatocyte invasion. Furthermore, the cost of producing a soluble CSP vaccine may be significantly lower than particulate vaccines. We have produced a nearly full-length CSP soluble protein vaccine (FMP013) in the *E. coli* system. In order to select an adjuvant for this vaccine, FMP013 was tested with a battery of adjuvants in the C57BL/6 mouse challenge model. We report here the immunogenicity and protective efficacy of the GMP FMP013 product with the Army Liposome Formulation (ALF) adjuvant. ALF contains immuno-stimulant 3D-PHAD (Avanti Polar Lipids) formulated in liposomes composed of phospholipids and cholesterol. Mice were immunized three times with a sub-saturating dose of CSP+ALF combined with either aluminum hydroxide (ALFA) or QS-21 (ALFQ). Immunogenicity and protection of the ALFA and ALFQ adjuvants were compared to Montanide ISA 720 adjuvanted FMP013. Mice were challenged with transgenic *P. berghei* sporozoites expressing *P. falciparum* CSP two weeks after final immunization. Especially high levels of protection (up to 100%) were observed in the CSP+ALFQ groups; this correlated with significantly higher CSP and NANP-specific antibody titers. ALFQ groups also showed higher IgG2c titers and a TH1-biased IgG2c:IgG1 ratio. Enhanced signs of early

B-cell development and germinal centers were observed in the ALFQ groups compared to the Montanide group. Initial findings suggest that soluble CSP and ALFQ combination may hold promise. These data are now being confirmed in the Rhesus model, which will be the final go-no-go decision point For advancing this vaccine into humans.

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MASS DIRECT SKIN FEEDS OF *ANOPHELES COLUZZII* IN THE CONTEXT OF MALARIA TRANSMISSION BLOCKING VACCINE TRIALS IN BANCOUMANA, MALI

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As proxy measures to assess the activity of malaria transmission blocking vaccines (TBV), standard membrane feeding assay (SMFA), direct membrane feeding assay (DMFA), and direct skin feeds (DSF) were previously implemented only at small scale. Here we investigate the performance of mass DSF in Bancoumana, Mali. From September to November 2015, we prepared each week ~30,000 laboratory reared female *Anopheles coluzzii* 3-8 days of age, and conducted twice-weekly feeding assays for 6 weeks on ~200 volunteers aged 18-50 years participating in Pfs25-Pfs230 TBV trials. Thirty starved mosquitoes in each of two cups were fed on the arms of volunteers for 15 minutes. Fed mosquitoes were dissected for *Plasmodium falciparum* oocyst counts. Of 119,220 mosquitoes the feeding rate was 97% and the survival rate of fed mosquitoes was 75%. Of 87,487 dissected mosquitoes, the overall infection rate was 0.5% resulting from 58/2008 DSF assays (2.5%). During the 6 weeks of DSF assays, weekly infection rates varied from 0.1% to 1.1%. Among infected mosquitoes, 78% had loads between 1-10 oocysts per midgut, 7% had between 11-20 oocysts, and 15% had more than 20 oocysts. Of all DSF assays performed, greater than mild adverse events were only observed in one individual, and resolved with topical treatment. The results demonstrate the feasibility and safety of mass DSF for implementation in TBV trials, and provide a basis to calculate sample sizes for this community using mosquito infection as an endpoint.

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DESIGN, EXPRESSION AND SCALABLE CGMP PRODUCTION OF FMP014 - A SELF-ASSEMBLING PROTEIN NANOPARTICLE AS THE BASIS OF A VACCINE AGAINST *PLASMODIUM FALCIPARUM* MALARIA

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In spite of the best efforts of the global research community only a handful of vaccine candidates have shown promise in combating malaria and most of these vaccines have been presented as live attenuated viruses or as virus-like particles. We have developed FMP014, a vaccine against *Plasmodium falciparum* malaria, which is comprised of 60 identical protein chains that form a small icosahedral shaped self-assembling protein nanoparticle (SAPN) similar to the capsid of small viruses. Each monomer displays selected *P. falciparum* Circumsporozoite Protein (PfCSP) CD4 and CD8 epitopes, universal TH epitopes, and portions of the α-TSR domain and NANP repeats of the PfCSP. Here we describe the conditions that are required for successful scale-up and cGMP manufacturing of FMP014. Furthermore, we demonstrate that when assembled and formulated with the Army Liposomal Formulations ALFA, ALFQ or ALFQA the nanoparticle vaccine prevents infection of mice by an otherwise lethal dose of transgenic *P. berghei* sporozoites expressing the complete PfCSP. (In depth analysis of the humoral and cellular responses to FMP014 are given in accompanying posters by Kaba et al. and Storme et al.). The cGMP SAPN and ALF adjuvants are currently undergoing studies in nonhuman primates and will be further tested in human volunteers in 2017.

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OVERCOMING DIVERSITY OF AMA1: EVIDENCE OF POLYMORPHISM DILUTION MEDIATED REFOCUSING OF RESPONSES TOWARDS CONSERVED EPITOPES IN RHESUS MONKEYS VACCINATED WITH QUADVAX+AS01

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Vaccines against polymorphic antigens require strategies that cover the entire antigenic spectrum of prevailing strains in an endemic area. Given the extreme diversity of Apical Membrane Antigen-1 (AMA1) of *Plasmodium falciparum*, the down-selection of strains for a polyvalent vaccine remains a challenge. We propose that QuadVax (a mixture of only four AMA1 strains: 3D7+FVO+HB3+W2mef) may provide global coverage as was observed by *in vitro* GIA using rabbit antibodies to QV generated using Montanide ISA720 adjuvant (Dutta et al. PLOS Pathogens 2013). We now report results of a Rhesus trial that was conducted to determine if observations made originally in the rabbit model using Montanide adjuvant would hold up in the Rhesus model using QV formulated in a human-use adjuvant AS01 (GlaxoSmithKline). Two groups of Rhesus (n=7) received three immunizations of 20 ug (LO group) or 80 ug (HI group) monovalent 3D7 AMA1 in AS01 and two comparator groups received 20 (LO) or 80 ug (HI) QV in AS01. There was no significant difference between the immunogenicity and GIA activity of the LO vs. HI dose groups. Both the 3D7 and QV vaccinated animals induced high levels of ELISA and GIA activity against the homologous 3D7 strain parasites. In heterologous ELISA and GIA the two QV groups (HI and LO) showed significantly higher cross-reactivity and heterologous GIA compared to the two 3D7 AMA1 groups. The original observations of strain-broadened responses by QV in rabbits were reproduced whereby QV vaccination shifted the immune response towards cross-reactive epitopes on the domain-3 and conserved face of AMA1. Furthermore increased responses to the conserved regions correlated positively with higher cross-strain GIA. Our data pave way for continued development of AMA1 as a malaria vaccine. The concept of polymorphism dilution by mixing a small number of antigenically diverse strains may be applicable to developing pan-reactive vaccines against other diverse pathogens like dengue, HIV and influenza.

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T CELL IMMUNOGENICITY AND CORRELATES OF PROTECTION FROM A DOSE-ESCALATION SAFETY AND EFFICACY STUDY OF PFSPZ WITH CHLOROQUINE IN MALARIA-NAÏVE ADULTS

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High-level protective efficacy is induced in a dose-dependent manner by infectious PfSPZ administered under chloroquine prophylaxis (Pf-CVac) followed by controlled human malaria infection (CHMI). Here we present the T cell responses after three immunizations with 3.2x10^3, 1.28x10^4, and 5.12x10^4 PfSPZ at 4-week intervals while taking chloroquine. The memory phenotype and effector function of T cell responses were assessed by 14-16-color multi-parameter flow cytometry after the final immunization and at the time of homologous CHMI. Pf-specificity was determined by incubating PBMCs with aseptic PfSPZ, vaccine diluent (HSA), Pf-infected erythrocytes (PfRBC), or uninfected RBCs. The staining panel included CD4, CD8 and γδ T cells, chemokines, activation markers, and the cytokines IFN-γ, IL-2, TNF-α, IL-4, and IL-10. The magnitude of Pf-specific Th1 cytokine-producing CD8, CD4, and γδ T cell responses following Pf-CVac were dose-dependent. A high frequency of PfRBC-specific memory CD4 T cells (median of 1.6%) was detected after final immunization in the highest dose group of 5.12x10^4 PfSPZ. In an exploratory analysis, immune responses that correlated with protection were assessed using a stratified Wilcoxon test controlling for vaccine dose. The magnitude of PfRBC-specific memory CD4 T cells simultaneously expressing IFN-γ, IL-2, and TNF-α correlated with protection with an uncorrected P-value of 0.00043. These findings demonstrate that Pf-CVac induced high-magnitude cytokine-producing T cells in multiple effector lineages in a dose dependent manner and provide evidence that PfRBC-specific multi-functional memory CD4 T cells may be a correlate of protection. Establishing such responses as a correlate of protection will require validation in larger prospective studies.

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EQUATORIAL GUINEA'S FIRST EVER CLINICAL TRIAL: TOLERABILITY, SAFETY AND IMMUNOGENICITY OF PFSPZ VACCINE IN YOUNG EQUATOGUINEAN ADULTS

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PfSPZ Vaccine is a candidate pre-erythrocytic malaria vaccine composed of radiation-attenuated, aseptic, purified, cryopreserved *Plasmodium falciparum* (Pf) sporozoites (SPZ). In clinical trials in the U.S. and Africa, PfSPZ Vaccine administered by direct venous inoculation (DVI) provides durable protection against heterologous strains and heterogeneous populations of Pf. Trials in young children are underway. A robust malaria control program has substantially reduced the malaria burden on Bioko Island, Equatorial Guinea (EG). With an eye toward eventual elimination of malaria on Bioko, a malaria vaccine initiative was established to evaluate the potential utility of PfSPZ Vaccine, resulting in the first ever clinical trial in EG. This first trial was a phase 1, randomized, double blind placebo-controlled trial to assess the tolerability, safety, and immunogenicity of PfSPZ Vaccine administered by DVI in young, healthy 18-35 year olds. A sentinel group of three volunteers received an initial PfSPZ Vaccine dose of 1.35x105 PfSPZ and a second dose of 2.7x105 PfSPZ was given two weeks later. Following review by the safety monitoring committee, 30 volunteers were randomized to receive three doses of either 2.7x105 PfSPZ or normal saline placebo at 0, 8 and 16 weeks. Adverse events (AEs) were solicited for 7 days after each vaccination. There was enhanced surveillance and reporting for unsolicited AEs for 28 days after each vaccination. Monitoring for severe adverse events was done throughout the 40-week study period. Blood samples for safety monitoring of hematological, renal and hepatic functions were taken at baseline, 2, 7, 14 and 28 days after each vaccination and monthly after the last dose for six months. Blood samples for antibody and cellular immunology endpoints were taken at baseline and 1 month after the last vaccination. The results of safety, tolerability and immunogenicity will be presented, including a comparison of the immunological response to the same dose of PfSPZ in EG and Tanzania.

1646

PLACENTAL MALARIA VACCINES: COMPARING GLYCOSYLATED AND NON-GLYCOSYLATED N-TERMINAL DOMAINS OF *PLASMODIUM FALCIPARUM* VAR2CSA PROTEIN PREPARED AS RECOMBINANT PROTEIN OR DNA VACCINES

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Placental malaria (PM) causes poor pregnancy outcomes including severe maternal anemia and low birth weight, but adverse outcomes decrease over successive pregnancies as women acquire immunity. PM is caused by parasites that express the PfEMP1 variant antigen VAR2CSA and bind the placental receptor chondroitin sulfate A (CSA). With increasing parity, women acquire anti-VAR2CSA antibodies and serum activity that inhibits adhesion of infected erythrocytes to CSA. Naturally acquired protection suggests that a vaccine against PM is feasible. While multiple VAR2CSA domains bind CSA in *in vitro* assays, earlier work showed the N-terminal fragment binds CSA with similar kinetics as the full length protein. We expressed glycosylated and non-glycosylated versions of two Pf FCR3 VAR2CSA N-terminal regions NTS–DBL1X–ID1–DBL2X and ID1–DBL2X–ID2a in insect cells as secreted proteins. We purified the histidine-tagged recombinant proteins from culture media using Ni Sepharose excel medium. In parallel, we generated DNA vaccine constructs using the same boundaries. Immunization studies using these proteins and DNA vaccine constructs have been initiated in rats. We will report the effect of DNA versus protein vaccinations as well as glycosylation status on the CSA binding inhibition activity of the resulting antibodies.

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CHANGING THE PARADIGM OF VACCINE DEVELOPMENT: FROM A WESTERN-LED TO AN INTERNATIONAL, MULTI-PARTNER, PARTIAL AFRICAN-FUNDED CONSORTIUM APPROACH

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Publication of the first PfSPZ Vaccine trial (Epstein et al., Science. 2011 334:475-80) offered clear scientific rationale to continue clinical development of *Plasmodium falciparum* (Pf) sporozoite (SPZ) products. In the absence of major continued funding, the International PfSPZ Consortium (I-PfSPZ-C) was established to design and execute a series of independently funded clinical studies of PfSPZ Vaccine and PfSPZ Challenge (infectious PfSPZ) by partner institutions. I-PfSPZ-C members have finalized the dose and route of administration of PfSPZ Challenge and used PfSPZ Challenge as a unique clinical research tool to understand malaria biology in 18 controlled human malaria infection (CHMI) studies in the US, and Europe and Africa. Members have completed clinical trials of PfSPZ Vaccine and PfSPZ-CVac in the US (N=4), Europe (N=3) and Africa (N=3), are currently or imminently conducting 12 new studies, and are developing new partnerships in Asia. Intravenous immunization with PfSPZ can repeatedly induce high level protection against CHMI and natural malaria exposure. Studies developed under a Clinical Development Plan are reviewed by the I-PfSPZ-C, their cross-site adaptive design being used to inform ongoing and future trials. The I-PfSPZ-C has remained inclusive - individuals, groups, institutions and funding organizations participate in reviewing all published and unpublished data, and planning new trials and strategies. The I-PfSPZ-C meeting at ASTMH Philadelphia 2015 included 104 participants from 38 organizations in 18 countries, all with independent funding. Collaborating I-PfSPZ-C members provide essential planning and governance for PfSPZ Vaccine and PfSPZ-CVac licensure. Uniquely, there has been significant investment by African governments; first by the Tanzanian Commission on Science Technology, and the second and largest ($48.5M) by the Government of Equatorial Guinea (75%) and 3 private energy partners (25%), plus $10M from the Government of Gabon. The I-PfSPZ-C has changed the paradigm of vaccine development from a western driven effort, to one funded and led by all partners, and uniquely those in Africa.

1648

ADVANCING PARASITOLOGY, ENTOMOLOGY AND VACCINOLOGY BY MANUFACTURING WHOLE PARASITE (EUKARYOTIC CELL) ASEPTIC, PURIFIED VACCINES PRODUCED IN ARTHROPODS: CHALLENGES, SUCCESSES AND TRAJECTORY FOR PFSPZ VACCINES

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There are no 1) vaccines for parasitic diseases, 2) preventative vaccines composed of eukaryotic cells, or 3) vaccines produced in mosquitoes that are licensed by the FDA. Thus, when Sanaria proposed producing *Plasmodium falciparum* (Pf) whole parasite sporozoite (SPZ) (eukaryotic cell) vaccines in aseptic mosquitoes to prevent malaria, most thought this would be impossible. When we began there were no methods to, 1) produce aseptic mosquitoes or aseptic PfSPZ in these mosquitoes, 2) aseptically harvest the parasites from mosquitoes, 3) adequately purify PfSPZ from the mosquito material, 4) stabilize these eukaryotic cells, 5) do this in compliance with regulatory standards (GMPs), or 6) at a scale adequate to support commercial launch in a facility that cost less than $4M. During the last decade we have accomplished all of this, and demonstrated that the PfSPZ are potent: 3 doses of a PfSPZ-based vaccine provides the highest durable protection ever demonstrated against controlled human malaria infection and exposure to malaria in the field. In double blind, placebo-controlled trials there have been no differences in adverse events between vaccinees and controls validating that the PfSPZ are pure. Trials to finalize immunization regimens for phase 3 trials and licensure are underway. We now produce in the same facility and with the same staff, >7 fold more PfSPZ in a single day than we did during the manufacturing campaigns that supported our first 18 clinical trials conducted from 2009 to 2014. Creating this disruptive technology has required, 1) ignoring conventional approaches to manufacturing and storing vaccines, 2) enormous attention to establishing highly efficient manufacturing teams and transparent communication with regulatory authorities, and 3) never forgetting that licensure and deployment of a highly effective vaccine for those most in need in Africa is our raison d'etre. We believe the principles and techniques Sanaria has followed/established leading to this success may be of technical and inspirational value to those working to produce vaccines against other parasites and arthropod diseases, and these will be presented.

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DISTRIBUTION LOGISTICS TO SUPPLY CRYOPRESERVED PFSPZ VACCINE TO TRAVEL CLINICS AND MALARIA MASS IMMUNIZATION PROGRAMS

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Sanaria's *Plasmodium falciparum* (Pf) sporozoite (SPZ)-based PfSPZ Vaccine, PfSPZ Challenge and PfSPZ-CVac, are live whole organism products thermostabilized by cryopreservation, stored and distributed in liquid nitrogen vapor phase (LNVP) using LNVP dry shippers. LNVP storage imparts extreme stability compared to conventional vaccines' fridge or freezer storage, and provides many additional advantages. The LNVP cold chain has supplied 18 PfSPZ clinical trials to date and an additional 11 trials in progress (or commencing during 2016) in the USA, Europe and Africa. Vaccine is packaged in cryovials: until now, closure of these cryovials has been by standard cryovial screw caps necessitating vaccine thawing/reconstitution and syringe preparation in a biological safety cabinet (BSC) at the clinic. A revolutionary new design of cryovial with tamper-evident closure and integral co-molded septum allows for immunizations anywhere without need of a BSC. A new custom dry thawing device obviates the need for a water bath for cryovial thawing. A new high density packaging system delivers 1,300 cryovials per standard dry shipper (or 200 per backpack dry shipper for last mile) to immunization clinics. The highly efficient hub-and-spoke (H&S) distribution system is used for distributing PfSPZ directly from Sanaria to immunization sites without intermediate stops; dry shippers also provide the local on-site storage. For travel medicine clinics in the USA, Europe and Japan H&S distribution will incorporate reverse logistics to rotate dry shippers on a 2-4 week schedule of restocking and resupply, augmented where appropriate, with extended local LNVP or Stirling freezer storage at immunization clinics and be coordinated by a third party logistics company. In malaria endemic regions, scale up first to Phase 3 trials and then to implementation in mass immunization programs for malaria elimination, distribution will utilize the H&S system operating from major regional storage hubs. In its scope and scale, this application of LNVP cold chain logistics will become a new operational standard.

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EVALUATION OF RPFMSP2-BASED VACCINES FOR INCLUSION IN A MULTI-COMPONENT MALARIA VACCINE FORMULATION

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Naturally-acquired antibody responses to Merozoite Surface Protein 2 (MSP2) in individuals living in *Plasmodium falciparum* endemic areas are associated with resistance to malaria. Earlier vaccine trials showed that

immunization-induced *Pf*MSP2 specific antibodies correlated with partial protection against *P. falciparum* malaria. These naturally-acquired and vaccine-induced anti-*Pf*MSP2 antibodies are primarily directed against the central variable region of two major allelic variants of *Pf*MSP2. These data suggest that *Pf*MSP2 has significant potential as a vaccine candidate, likely as a component of a multiantigen formulation. In prior studies with multivalent blood-stage antigen formulations, we showed that genetic fusion of *Pf*MSP1$_{19}$ to the N-terminus of MSP8 facilitated vaccine production, minimized antigenic competition and markedly enhanced induction of functional antibodies. To further improve protective efficacy, we are working to formulate *Pf*MSP2 in combination with the *Pf*MSP1/8 candidate. A synthetic *Pf*MSP2 (3D7) gene, codon-harmonized for expression in *E. coli*, was used to produced r*Pf*MSP2 as an unfused full-length protein or as a chimeric antigen linked to the N-terminus of *Pf*MSP8. Purification of r*Pf*MSP2 and chimeric r*Pf*MSP2/8 yielded 29 mg/L and 54 mg/L of bacterial culture respectively. Vaccine-induced anti-*Pf*MSP2-specific antibodies were quantitated following immunization of CB6F1/J mice with unfused *Pf*MSP2, chimeric *Pf*MSP2/8, or an admixture of r*Pf*MSP2 and r*Pf*MSP8, each formulated with Alhydrogel as adjuvant. High and comparable anti-MSP2 antibody titers were elicited by immunization with the expected predominance of Th2-biased antibody isotypes. Of significance, no evidence of competition between component antigens was noted. Sera from immunized animals strongly recognized native MSP2 in both immunoblot and indirect IFA of *P. falciparum* blood-stage parasites. Comparative assessment of vaccine-induced B and CD4+ T cell responses and antibody-mediated merozoite neutralization will inform selection of a highly immunogenic *Pf*MSP2 antigen to be formulated in combination with *Pf*MSP1/8.

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USE OF A *PLASMODIUM*-SPECIFIC CARRIER PROTEIN TO ENHANCE PRODUCTION OF RECOMBINANT PFS25, A LEADING TRANSMISSION-BLOCKING VACCINE CANDIDATE

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Despite reductions in morbidity and mortality worldwide, eradication of *Plasmodium falciparum* malaria will most likely require a multi-stage, multi-antigen vaccine that incorporates a target of transmission-blocking antibodies. *Pfs*25 is one such transmission-blocking vaccine candidate. Antibodies directed against conformational epitopes within the highly constrained EGF-like domains of *Pfs*25 block sexual stage development in mosquitoes. However, the large-scale production of properly folded recombinant *Pfs*25 has been challenging. We have previously shown that use of *Pf*MSP8 as a fusion partner for *Pf*MSP1, facilitated production of properly folded *Pf*MSP1$_{19}$ and promoted induction of high titers of growth inhibitory antibodies upon immunization. Here, we applied a similar strategy for the production of a *Pfs*25-based vaccine. The gene for *Pfs*25 was codon-harmonized for expression in *E. coli* SHuffle^{Tm} T7 Express *lysY* cells. Recombinant *Pfs*25 was produced as a single, unfused antigen (u*Pfs*25) or chimeric *Pfs*25-*Pf*MSP8 fusion protein (c*Pfs*25/8). uPfs25 was purified under denaturing conditions with subsequent refolding with a yield of ~8.4 mg/L of bacterial culture. The chimeric *Pfs*25/8 was successfully purified under non-denaturing conditions with a yield of 43 mg/L, highlighting value of the *Pf*MSP8 carrier. Proper folding of these antigens was verified by SDS-PAGE under reducing vs non-reducing conditions and by immunoblot analysis with mAb 4B7 which recognizes a conformational epitope of *Pfs*25. Antisera against both antigens were produced in NZW rabbits and assessed for titer and functionality. Both antigens induced strong and comparable titers against *Pfs*25 which potently inhibited parasite transmission to mosquitoes in a standard membrane feeding assay. Although *Pf*MSP8 is also expressed in gametocytes, rabbit anti-*Pf*MSP8 antibodies did not block transmission. Comparative immunogenicity studies are currently underway to further assess the humoral and cellular response to these antigens, to aid in selection of a highly efficacious *Pfs*25 based vaccine to include in a multi-antigen, multi-stage vaccine formulation.

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ANTIBODY PROFILES TO WHEAT GERM CELL-FREE SYSTEM SYNTHESIZED *PLASMODIUM FALCIPARUM* PROTEINS CORRELATE WITH PROTECTION FROM SYMPTOMATIC MALARIA IN UGANDA

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The key targets of protective antibodies against *Plasmodium falciparum* remain largely unknown. The aim of this study was to identify proteins whose antibodies are correlates of malaria acquired immunity that could be relevant for development as malaria vaccine candidates. We profiled immune responses to 1827 wheat germ cell-free system (WGCFS) expressed proteins derived from 1586 genes representing ~30% of the *P. falciparum* entire genome. We previously reported that several WGCFS expressed *P. falciparum* proteins could elicit antibodies in immunized animals that exhibited growth inhibition activity *in vitro*, suggesting that the recombinant proteins, at least in part, retain their natural conformations. Serum samples were obtained from individuals aged 6-20 years who are indigenous residents of a malaria holoendemic community in Northern Uganda. They were enrolled at the start of the rainy season and prospectively monitored for clinical malaria episodes for one year. Protein immunoreactivity to serum samples was determined by AlphaScreen; a homogeneous high-throughput system to detect protein interactions. More than 51% (936/1827) of the proteins reacted with the sera. Subsequently, antibody levels to 9 proteins, encoded by 8 genes significantly associated with time to the first symptomatic malaria episode in children. The 9 proteins comprised both previously characterized vaccine candidates and novel uncharacterized proteins. WGCFS combined with AlphaScreen offer an alternative approach to genome-wide screening of malaria antigens associated with acquired immunity.

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IDENTIFICATION OF PFRIPR, AN RH5-INTERACTING PROTEIN, AS A HIGHLY CONSERVED BLOOD-STAGE MALARIA VACCINE CANDIDATE AGAINST *PLASMODIUM FALCIPARUM*

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Blood-stage malaria vaccine (BSV) candidates of high efficacy against *Plasmodium falciparum* (Pf) remain elusive, mainly because of genetic diversity and allele-specific immunity in endemic regions. Here, we hypothesize that in order to improve efficacy, BSV candidate antigens should contain conserved targets of immunity. Hence, identification of suitable candidates for downstream clinical studies in the quest for next generation vaccines requires understanding of the extent of genetic polymorphisms in the antigen targets and the roles played in naturally acquired immunity. Using field isolates from Uganda, we carried out genetic analyses on genes encoding four recently reported novel BSV candidate proteins; RH5 interacting protein (PfRipr), GPI anchored

micronemal antigen (PfGAMA), rhoptry-associated leucine zipper-like protein 1 (PfRALP1) and Duffy binding-like merozoite surface protein 1 (PfMSPDBL1). In addition, we expressed recombinant proteins of these candidates based on Pf laboratory clone 3D7 sequences using wheat germ cell-free system, immunized rabbits to obtain specific antibodies (Abs) and performed functional studies (Growth inhibition Assay, GIA). The GIA activity of the raised Abs in blocking erythrocyte invasion was determined using both the homologous 3D7 and heterologous FVO strains *in vitro*. *Pfgama* and *pfmspdbl1* are relatively polymorphic and Abs against the 3D7 recombinant PfGAMA and PfMSPDBL1 inhibited merozoite invasion of 3D7 but not FVO. Although both *pfripr* and *pfralp1* are conserved and Abs against their 3D7 recombinant proteins potently inhibited merozoite invasion of both 3D7 and FVO, the GIA activity of anti-PfRipr was much higher than that of anti-PfRALP1 on both 3D7 and FVO. Furthermore, *pfralp1* is comparatively diverse, with varied number of regions having insertions and deletions, asparagine and 6-mer peptide repeats in the sequences that are lacking in *pfripr*. Therefore, PfRipr is a highly conserved promising BSV candidate in the design of next-generation vaccines against *P. falciparum*.

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MULTIPLE INSECTICIDE RESISTANCE IN *AN* HIGHLY INFECTED POPULATION OF THE MALARIA VECTOR *ANOPHELES FUNESTUS* IN BENIN

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Knowledge on the spread and distribution of insecticide resistance in major malaria vectors such as *Anopheles funestus* is key to implement successful resistance management strategies across Africa. Here, by assessing the susceptibility status of an inland population of *An. funestus* (Kpome) and investigating molecular basis of resistance, we show that multiple resistance in this species now extends beyond the original coastal region of Benin and is associated with high infection rate. The TaqMan analysis of plasmodial infections revealed an unusually high infection rate (18.2%) of *An. funestus* in this locality. The WHO bioassays revealed a multiple phenotypic resistance profile for *An. funestus* in Kpome. This population is highly resistant to pyrethroids (permethrin and deltamethrin), organochlorines (DDT), and carbamates (bendiocarb). A reduced susceptibility was observed with dieldrin. Mortalities did not vary after pre-exposure to PBO for DDT indicating that cytochrome P450s play little role in DDT resistance in Kpome. In contrast, we noticed, a significant increase in mortalities when PBO was combined to permethrin suggesting the implication direct involvement of P450s in pyrethroid resistance. A high frequency of the L119F-GSTe2 DDT resistance marker was observed in this highly DDT resistant population (9%RS and 91%RR) whereas the A296S mutation was detected at a low frequency (1%RS and 99%SS). In conclusion, the extension to the inland locality of the multiple resistance in *An. funestus* populations suggests resistance could be widespread in Benin and this highlights the need for further studies to assess the geographical distribution of insecticide resistance across Benin and neighboring countries as well as a more comprehensive analysis of the resistance mechanisms involved. Keywords: Malaria, Benin, *An. funestus*, insecticide resistance, resistance mechanisms, malaria control.

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COVERAGE OF SEASONAL MALARIA CHEMOPREVENTION DELIVERED BY MOBILE TEAMS AT FIXED POINTS IN 14 DISTRICTS IN MALI, THROUGH ACCESS-SMC

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Mali was one of the first countries to introduce Seasonal Malaria Chemoprevention (SMC), starting in one district in 2012, and reaching 48 of the 63 districts in the country in 2015. We evaluated the effectiveness of SMC delivery in 14 districts where implementation was supported by the ACCESS-SMC project funded by UNITAID. SMC was administered four times at monthly intervals starting late August. Delivery was at fixed points by mobile teams who checked children for fever, administered SMC to those who were well, and tested those with fever with a malaria Rapid Diagnostic Test (RDT). RDT positive children were treated with artemether-lumefantrine, negative children received SMC drugs and if appropriate, an antibiotic. Most children could therefore be treated by the team rather than having to be referred to a health facility. Each child was issued with a three-year SMC record card on which the date of attendance, and whether the child was treated with SMC, or tested and treated for malaria, or excluded, was recorded. A survey was undertaken in December to assess coverage. 50 clusters (villages or quarters of urban areas) were selected with probability proportional to size in 5 districts. In each cluster about 20 children aged between 4 months and 7 years were surveyed. 1037 children were surveyed of whom 740 were eligible for all 4 SMC cycles because they were aged between 3 months and 5 years at the time of the first cycle. 85% of these children had received an SMC card. Based on the card, and on caregiver's recall if the card was not seen, 38% of eligible children who had received 4 SMC cycles. The main reasons for not receiving SMC were being away when the team came (50%); being unaware of when SMC teams would come (35%); and the caregiver being too busy (17%). Reported adherence to the two amodiaquine doses administered by the caregiver was 99% for each dose. A high proportion of children were reached at least once but, in this first year of national scale-up, less than half received the full number of cycles. Emphasis on sensitization of the community, and adoption of door-to-door distribution, may be needed to maximize the number of children protected.

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DESIGN, MONITORING, AND IMPLEMENTATION OF THE THIRD AND FOURTH ROUNDS OF SCHOOL NET DISTRIBUTION TO MAINTAIN UNIVERSAL ACCESS TO LONG-LASTING INSECTICIDAL NETS IN SOUTHERN TANZANIA

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In 2011, the Ministry of Health and National Malaria Control Program of Tanzania developed a Keep Up Strategy with the goal of maintaining the population's access to an ITN at or above 80%, by using school-based distribution as an innovative distribution channel. This strategy was piloted in the Southern Zone beginning in 2013, when the NMCP and the Tanzanian Red Cross Society distributed ITNs in 2,302 schools

in 19 districts in Lindi, Mtwara, and Ruvuma, a total of 421,285 ITNs, to classes 1,3,5,7 in primary school and Form 2 and 4 in secondary schools. By August 2016 Tanzania will have implemented four annual rounds of school-based distribution in three Southern regions. SNP2 was implemented in 2014 by NMCP and Research Triangle Institute, delivering 489,099 ITNs to school children, and adding classes 2 and 4 in Lindi. In the third round in 2015, NMCP with JHUCCP's VectorWorks project delivered 494,407 ITNs to 1,919 schools in the 19 districts, targeting classes 1-3, 5, and 7 in primary school in Ruvuma and Mtwara, and classes 1-5 and 7 in Lindi. The 4th round in August 2016 will continue in the three regions in the south and expand to four regions in the Lake Zone; 1,310,000 ITNs will be delivered to 5,054 schools in a total of seven regions. Working with a multi-sectoral task force including Ministry of Health, Ministry of Education, and local officials, enrolment data was gathered from each school, verified, and used to quantify deliveries for each school. After training and delivery of ITNs to schools, teachers distributed ITNs to the eligible students in the targeted classes, and provided behavior change messages on net use, care, and malaria prevention. We will discuss the design, implementation and outcomes of SNP3 and SNP4, including the process of quantifying the ITN needs, training and sensitization activities, data management, and logistics considerations for an ongoing, mass yearly distribution of nets to schoolchildren. We will also discuss changes from SNP1 to SNP4 in the operations management, in particular, decisions to adjust the number of classes targeted each year based on evaluation data, and implications for future national scale-up.

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PLANT-MEDIATED EFFECTS ON MOSQUITO CAPACITY TO TRANSMIT HUMAN MALARIA

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The ecological context in which mosquitoes and malaria parasites interacts has received little attention, compared to the genetic and molecular aspects of malaria transmission. Plant nectar and fruits are important for the nutritional ecology of malaria vectors, but how the natural diversity of plant-derived sugar sources affects mosquito competence for malaria parasites is unclear. To test this, we infected *Anopheles* coluzzi, an important African malaria vector, with sympatric field isolates of *Plasmodium falciparum*, using direct membrane feeding assays. Through a series of experiments, we then examined the effects of nectar from Thevetia neriifolia and Lannea microcarpa, and fruit from Barleria lupilina and Mangifera indica on parasite and mosquito traits that are key for determining the intensity of malaria transmission. We found that the source of plant sugar differentially affected infection prevalence and intensity, the development duration of the parasites, as well as the survival and fecundity of the vector. These effects are likely the result of complex interactions between toxic secondary metabolites and nutritional quality of the plant sugar source, as well as of host resource availability and parasite growth. Using an epidemiological model, we show that plant sugar source can be a significant driver of malaria transmission dynamics, with some plant species exhibiting either transmission-reducing or -enhancing activities.

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PROCESS EVALUATION OF CONTINUOUS ITN DISTRIBUTION IN ZANZIBAR

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In 2013, Zanzibar designed a continuous ITN distribution strategy to maintain high levels of ITN ownership and use. ITNs are given to pregnant women and caretakers of young children through free distribution at 1st ANC visit and 9 month measles vaccination, respectively. At the community level, households request a coupon from the sheha to redeem for a LLIN and then exchange the coupon at a health facility for a new ITN. Coupons are then returned to ZaMEP. In addition, coupons can be issued by district malaria surveillance officers during case investigations if LLIN need is identified. From June 2014 to January 2016, the Zanzibar Malaria Elimination Program (ZaMEP) reported that 289,661 ITNs were distributed through continuous distribution: 65,325 to pregnant women at ANC, 60,507 to caretakers at EPI clinics, and 163,829 through the community channel. A total of 40 semi-structured interviews took place in early April in a convenience sample of 8 shehias and 16 distribution points on Unguja and Pemba Islands, Central, West B, Chake Chake, and Mkoani districts. Interviews took place at distribution points for coupons and for LLINs, with central level stakeholders, and with health facility staff and shehas at community level to identify implementation bottlenecks and best practices. Record reviews of LLIN distribution, stock data, and costs were done at central, district, shehia, and health facility level. Ten observations of coupon/ITN redemption were made. Preliminary findings indicate that overall, the CD channels are functioning, with challenges in stockouts of both ITNs and coupon-books at certain health facilities and shehias, and in adequate storage facilities for ITNs at health facilities. Stakeholders at all levels felt the program has made a positive contribution to malaria control, but there is a need for clarity in reporting systems and increased supportive supervision and refresher training, particularly at the shehia level. Additional findings, including estimates of ITN ownership and access resulting from the continuous distribution, and cost per-ITN-distributed, will be presented after the data is fully processed and analyzed.

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ASSESSMENT OF MALARIA TRANSMISSION FROM HUMAN TO MOSQUITOES IN SEASONAL MALARIA CHEMOPREVENTION IN THE WESTERN REGION OF BURKINA FASO

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Seasonal malaria chemoprevention (SMC) can reduce malaria cases up to 80% in sahelian region. However the impact of SMC on human to mosquito malaria transmission is currently unkown. Here, we evaluated the infectiousness to mosquitoes of volunteers receiving SMC by membrane feeding assays. Children over the age of 2 years, participants of a SMC clinical trial were randomly selected. They were invited to participate after

a clinical examination and irrespective of their parasite carriage status. Blood sample were collected in 5 sites (4 under SMC treatment and 1 control) over a period of 4 months from August to November 2015. In total 301 children were involved, 204 children in SMC group and 77 in control group. For each blood sample, 80 female *Anopheles* mosquitoes were provided a blood meal through a parafilm membrane. On day 7 after feeding, mosquitoes were dissected and midguts were screened for the presence of oocysts. Generalized linear mixed models were used to compare mosquito infection in treatment and control group and to estimate intervention efficacy. Results showed that gametocytemia was lower in the SMC groups with respect to the control: month 1 (X22=6.14, p=0.046), month 2, 3, and 4 (X21=57.3, p<0.0001). There was a strong impact of SMC on both oocyst prevalence with a 93 % reduction in mosquitoes that received blood from SMC patients (X22=182, p<0.0001), and oocyst density with a 86% reduction (X22=70.6, p<0.0001). In conclusion, in an area of seasonal malaria transmission, chemoprevention highly reduces human to mosquitoes malaria transmission.

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COMMUNITY ENGAGEMENT AROUND THE IMPLEMENTATION OF TRIAL OF INSECTICIDE-TREATED WALL LINING FOR MALARIA CONTROL IN RURAL TANZANIA

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Community engagement (CE) during community trials is a complex social phenomenon that defies simple explanation or mechanization. We present findings from an assessment of the sensitization process, experiences, and challenges in improving understanding and subsequent acceptance of an insecticide-treated wall lining (ITWL) program. The initial project sensitization plan relied on the traditional approach of inviting villagers to meetings with researchers. However, meeting schedules coincided with farming activities and Tanzania's presidential elections, resulting on poor attendance. Sensitization was re-strategized to add door-to-door sensitization using local advocates, announcements using a megaphone, and designing and distributing brochures detailing the study objectives and consenting process. The process continued during the ITWL installation phase. Following re-strategizing of sensitization, the ITWL acceptance rose to 86.4%. However, some clusters still had some refusals. Reasons included gender and consent, for example, in some houses the head of house (generally a man) refused installation after the wife had accepted. Old rumors resurfaced that ITWL contributed to male impotence. Some installers, initially unprotected, developed skin rashes. In one case, one resident's skin rashes spread fear to a whole hamlet. Households with better socio-economic status cited personal ability to control malaria and feared damage to their walls by the installation process. Directives that children should not touch the wall liners and confusion from installation delays all fed into refusal rates. Rumors of side effects from the ITWL contributed much on project challenges including refusals. Re-strategizing sensitization plus continuous sensitization throughout and after the official installation period increased ITWL acceptance. Future projects should incorporate continuous sensitization and consider using specialized village research committees for improved CE.

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SMALL SOLAR POWERED 'BɔKɔ' FANS IMPROVE COMFORT INSIDE MOSQUITO NETS IN SOUTHERN GHANA

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In rural Greater Accra, in 2014, 49% of people didn't use mosquito nets despite having access to a space under one. Discomfort due to heat is the most stated reason, but this problem is largely unaddressed. With advancing electrification and dropping price of solar power, 'Bɔkɔ' 0.8 W net fans equipped with a 0.1 W LED could improve comfort inside nets and be affordable to populations in malaria endemic areas. Ninety-two households (HHs) from rural communities in Greater Accra, divided into three groups, participated in a 10-month randomized cross-over trial, where fan systems (one fan per HH member in Group 1) were crossed over with water filters between Groups 1 and 2, while Group 3 served as control. Intervention HHs participated in fortnightly surveys on HH's practices related to mosquito nets, fans and water filters, while control HHs were questioned only at start, mid-point and study end. Further, key-informant interviews were held before mid-point (cross-over), and willingness to pay for fans was assessed in individual auctions at study end. Baseline net use conditional on access in the study communities was unexpectedly high at 92, 93, and 87% for Groups 1, 2 and 3, respectively, and increased to 99 and 99% at cross-over and 97 and 90% at end-point in intervention Groups 1 and 2, respectively, while it reduced to 81 and 84% in the control Group 3 at cross-over and end-point, respectively, indicating a Hawthorne / study effect. Stated fan use was 88-100% depending on the fortnight of survey. The main reason for using fans was heat, but it was also mentioned that they drove mosquitoes away. Key informants suggested they slept less exposed outside due to the fan during part of the night during the dry season. Despite the low power rating, nine out of 13 key informants stated that they placed the fan outside the bed net explaining that the air produced by the fan was enough to reach them through the net. The average bid price per fan was GH₵ 55 (~US$ 13.5), and in total 98 Bɔkɔ fans were sold to participating HHs. Small electric fans were accepted and desired in the study community and may be an affordable innovation to improve comfort inside mosquito nets in hot climates.

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DIHYDROARTEMISININ-PIPERAQUINE AS INTERMITTENT PREVENTIVE TREATMENT FOR MALARIA IN *A* REFUGEE CAMP, ADJUMANI, UGANDA

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An intermittent preventive treatment (IPT) program using dihydroartemisinin-piperaquine (DP) was implemented between March and July 2015 in a refugee camp in Adjumani District, Uganda. To our knowledge, this is the first implementation of IPT in the setting of a humanitarian emergency. Weight-dosed DP was offered to all children aged 6 months-14 years in the camp in March, May, and July 2015, at eight-week intervals. On average, 13 537 children received each distribution. To evaluate malaria incidence, reported cases were compared to the same 6-month period from 2014 taking into account population changes. To evaluate malaria prevalence, in the week prior to each

distribution and 8 weeks following the final distribution, malaria surveys were conducted in the camp. Thick and thin smears were collected from a target sample size of 250 persons in each of three age groups: <5 years; 5-14 years; ≥15 years. Direct microscopy was performed. In 2014, among children <5, malaria incidence was 0.71 cases/person over the 6 month-period running from March-August; in 2015 it was 0.52 (IRR 0.73, 95%CI 0.69-0.77) over the same period during IPT implementation. In children aged 5-14 years, the incidence was 0.96 cases/person in 2014 and 0.67 in 2015 (IRR 0.70, 95%CI 0.67-0.72). For those >15 years, the incidence was 0.37 cases/person in 2014 and 0.55 in 2015 (IRR 1.49, 95%CI 1.42-1.56). Among children <5, the prevalence of parasitemia by microscopy was 5.1% (95%CI 3.0-8.5) at baseline and 15.1% (95%CI 12.1-18.7) two months following the final distribution of DP. Among children aged 5-14 years, these figures were 8.7% (95%CI5.8-12.9) and 26.7% (95%CI 20.9-33.6), respectively. Among those over 15, the prevalences were 6.1% (95%CI 3.9-9.7) and 18.7% (13.7-25.0), respectively. In the setting of a humanitarian emergency, IPT reduced the incidence of malaria among its target population. Its impact may appear mitigated because of strong transmission seen at the end of the program in 2015, after the protective effect of DP had ended, as evidenced by the high incidence and prevalence seen in the untreated population.

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LLINS ASSESSMENT OF HOUSEHOLD COVERAGE IN DEMOCRATIC REPUBLIC OF CONGO BETWEEN 2004 AND 2014

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In DRC, malaria remains a major public health problem as the first cause of morbidity and mortality. To remedy this, the control strategies and approaches have been developed including support cases and vector control. The insecticide-treated net (LLIN) is a component of the vector control implementation in the country for over a decade. To obtain data that could reorganize malaria control, a critical analysis of the coverage level spaced ten years was performed. A community survey targeting the effective coverage of LLINs in households through the distribution and use of this material treated by communities (9 sentinel sites of NMCP). Cluster sampling was used for data collection. The statistical analysis took into account the 5% significance level. Between 2004 and 2014, the ownership of LLINs increased between 51.3% and 92%. As to the use, it was between 51% and 85% depending on the sites. The proportion of pregnant women sleeping under LLINs was between 61.7% and 89%. The protection of children under 5 years by the LLIN was between 57% and 91.6%. The sleeping places hedging average was 9.9% (3.4 to 16.4%, I.C 95%) in 2004, while coverage was 61.9% in 2014 (34.9 to 85%, I.C 95%). In conclusion, the universal coverage threshold was not achieved globally in the country. Efforts should be made to allow universal coverage nationally.

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ANOPHELES SUBPICTUS, A NEW DOMINANT MALARIA VECTOR IN AN URBAN AREA OF WESTERN INDIA

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Plasmodium and *Anopheles* in South Asia are under constant selection pressure due to ecological changes and parasite/vector control strategies of the Government of India. Most of the vector-parasite compatibility studies done in South Asia have centered around 6 major malaria vectors (*Anopheles culicifacies*, *An. fluviatilis, An. stephensi, An. minimus, An. dirus* and *An. sundaicus*), and these vectors are estimated to contribute to 95 - 98% of malaria cases. Much less is known about the role of other Anophelines in *Plasmodium* transmission in South Asia. There is a consistent increase in *P. falciparum* (Pf) cases in the last 40 years. As of December, 2015, Pf contributes to 67.5% of malaria cases in the Indian subcontinent. We hypothesize that, under intense drug and insecticide pressures, new parasite-vector associations could emerge based on the right physiological and phenotypic matches. In our US NIH International Centers of Excellence for Malaria Research (ICEMR) study site in Goa, Western India, a 2-year longitudinal study identified the mosquitoes that are naturally transmitting malaria in this region. *An. subpictus*, a previously overlooked minor vector, has emerged as a dominant malaria vector overtaking the primary vector, *An. stephensi*, and is transmitting malaria throughout the year. While in nature there are two sibling species of *An. subpictus* (A and B) in our study site, salivary gland infections were seen only in the sibling species, B. To facilitate larger surveys involving sibling species A and B of *An. subpictus*, a multiplex PCR assay has been developed. We have also colonized *An. stephensi* and have successfully performed laboratory infection experiments with patient blood containing *P. vivax* and *P. falciparum* infected patient blood. We describe efforts to colonize *An. subpictus* and compare the vector competence of *An. subpictus* and *An. stephensi* through controlled feeding experiments.

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COST-EFFECTIVENESS OF INSECTICIDE-TREATED WALL LINER AND INDOOR RESIDUAL SPRAYING TO PREVENT MALARIA IN KENYA AND TANZANIA

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Despite widespread distribution of long lasting insecticide bed nets (LLINs), malaria continues to be a major cause of morbidity and mortality globally. Indoor residual spraying (IRS) has proved efficacious, but is expensive, logistically complex due to needed reapplication every 6-12 months, and challenging due to insecticide resistance. A new technology, the non-pyrethroid insecticide treated wall liner (ITWL), may provide 3-4 years of protection from a single installation. Its two insecticides with different modes of action should curb insecticide resistance. We computed the incremental cost-effectiveness ratios (ICERs) of ITWL and IRS as supplements to LLINs in Kenya and Tanzania. One cluster randomized trial of the previous pyrethroid ITWL as a supplement to LLINs was conducted in Kenya. A similar trial (with the non-pyrethroid ITWL) is underway in Tanzania. We obtained acceptance rates, financial costs to the health

system, and effectiveness of IRS and ITWL through original data collection and available literature. Costs covered outreach, projected procurement and installation. We incorporated savings in medical costs from fewer malarial episodes. Estimated ITWL acceptance rates averaged 98% in Kenya (where extensive prior meetings with village leaders occurred) and 68% in Tanzania (where outreach began later). Average inpatient and ambulatory malaria episodes cost $59.80 and $8.06 in Kenya and $212.12 and $23.17 in Tanzania, respectively. In Kenya, one-time ITWL costs were $64.23 per person in 2010 while IRS cost $3.16 per person annually. In Tanzania, annual IRS costs were $15.59 per household in the target areas. Good communications proved critical to acceptance of ITWL. In Kenya, the ICERs were $482 per discounted life year gained (assuming protection lasts 3 years) for ITWL and $139 for IRS. As these ICERs were below Kenya's GDP per capita ($795), both technologies are highly cost-effective based on World Health Organization criteria. Although the ICER of ITWL was initially less favorable than that for IRS in Kenya, falling prices of LLINs suggest ITWL should also likely become less costly and more cost-effective.

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LAND COVER DETERMINANTS OF *PLASMODIUM FALCIPARUM* PREVALENCE IN URBAN AND PERI-URBAN AREAS OF NORTHERN BIOKO ISLAND

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Beginning in 2015, the Bioko Island Malaria Control Project (BIMCP) adopted a strategic and targeted approach of indoor residual spraying (IRS) for malaria prevention. Evidence suggests that hotspots of malaria transmission to be targeted are best evaluated by finding increased exposure to infectious mosquito bites. Despite routine vector monitoring throughout the island, mosquito collection in urban and peri-urban areas of northern Bioko has been logistically challenging. Remote sensing and geographic information systems (GIS) are frequently used to explore associations between land use/land cover (LULC) and mosquito-borne diseases by functioning as a proxy for mosquito abundance, while spatial scan statistics are used to detect spatial clustering of malaria prevalence. Data on prevalence of *P. falciparum* parasitaemia were collected from a representative sample of 5,286 households throughout the island during the 2015 Malaria Indicator Survey. Seven LULC types were classified through supervised classification of remotely sensed high resolution satellite imagery. Areas at higher risk of transmission were evaluated using a Bernoulli purely spatial scan statistic. Spatial associations of LULC and *P. falciparum* parasitaemia were performed using geographically weighted logistic regression analyses to determine case environmental risk factors. Complete data were available for 19,666 individuals. Although several statistically significant clusters were detected, two clusters of excess risk appeared to have been driven by one to two households with several cases. The most likely spatial clustering of malaria prevalence was detected in peri-urban areas, particularly in the northwestern region of the island, which appears to be temporally heterogenous. Results of geographic weighted regression will be analyzed further.*Plasmodium falciparum* malaria prevalence is heterogeneous in space in this urban and peri-urban study area. Geographical and housing risk factors associated with prevalence will be explored further. This analysis improves the planning of IRS interventions targeting high-risk areas.

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ENTERIC PATHOGEN SURVEILLANCE IN CHILDREN AND ADULTS IN *A* CASE-CONTROL STUDY OF ACUTE DIARRHEA IN BATTAMBANG, CAMBODIA

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Diarrhea has continued to be a major public health problem in developing countries and surveillance for for a wide range of enteric pathogens is necessary to understand frequency of pathogens. Stool samples, demographic and clinical data were collected from cases and asymptomatic controls in children (< 5 years old) and adults seen at Battambang Referral Hospital, SvayPor Health Center and Military Hospital 5 located in Battambang, Cambodia from July 2014 - April 2016. Standard microbiology for stool culture, ELISA and PCR were applied to detect enteric bacteria, virus and parasites. Antimicrobial susceptibility testing (AST) was tested by using disk diffusion method. In children < 5 years old, 226 cases and 226 controls were enrolled in the study in which rotavirus (20%), norovirus (16%) and *Shigella* (9%) were detected significantly more in the cases than controls. Enteroaggregative *E.coli* (24%), *Salmonella* (17%), *Campylobacter* (11%) were found in relatively similar proportions of cases and controls. Of 57 adult cases and 54 controls, bacterial pathogens including *Vibrio* (7%) and *Campylobacter* (4%) were identified significantly more in cases than controls. Enteric viruses were infrequently detected among the adult population. AST demonstrated multidrug resistant *Shigella* as well as co-resistance to extended spectrum cephalosporins and fluoroquinolones. Additionally, fluoroquinolone resistant *Campylobacter* was found in 80% of the isolates. Continued surveillance will provide data on etiologic agents and antimicrobial resistance patterns that are critical for treatment guidelines, prevention and control of diarrheal disease in Battambang, Cambodia.

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VIRULENCE PROFILE OF ENTEROTOXIGENIC *ESCHERICHIA COLI* (ETEC) STRAINS ISOLATED FROM PERUVIAN CHILDREN

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Enterotoxigenic *Escherichia coli* (ETEC) is a major cause of diarrhea in children and travelers. The aim of this study was to determine the presence of virulence factors (VF) of ETEC strains isolated from Peruvian children. We analyzed a total of 205 ETEC strains previously isolated from two cohort studies in children <24 months of age in Lima, Peru. ETEC was identified by a multiplex real-time PCR for lt and/or st genes. The presence of sixteen colonization factor (CF) types, ST toxin subtypes (STh and STp), adhesins (Tia, TibA, EtpA), a GTPase (LeoA), an autotransporter (EatA), an enteroaggregative *E. coli* heat-stable enterotoxin (EAST1) and an *E. coli* common pilus (ECP) genes were evaluated by PCR. LT-positive (ETEC-lt) strains (99/205, 48%) were the most frequent, followed by strains with only ST (ETEC-st) (63/205, 31%) and strains positive for both LT and ST (ETEC-lt-st) (43/205, 21%). Among ST-positive strains (with or without LT) STh (estA+) was the ST-toxin type most frequently identified (64/106, 60%). The most common CFs were CS21 (34%), CS6 (20%), CS3 (15%), and CS1 (12%). Presence of at least one CF were more frequently detected in isolates from diarrheal than control samples (81% vs. 52%, p<0.001). Whereas CS6 (26% vs. 13%, p<0.001), CS5 (13% vs. 2%, p<0.01) and CS1 (16% vs. 6%, p<0.05) were more frequently detected from diarrheal than control samples, respectively. STh+ CS21

genotype (with or without other CFs) was the most prevalent among all strains (19%, 38/205). On the other hand, the most common nonclassical VF (other than CFs and LT/ST toxins) were ECP (89%), EAST1 (44%), EatA (42%) and EtpA (32%). EatA was significantly detected in ETEC isolates from diarrheal than control samples (53 vs. 27%, p<0.01). Strains positive to at least one nonclassical VF were more frequently detected in isolates from diarrheal than control samples (100% vs. 95%, p<0.05). Using a prototype vaccine (with LT toxoid, CFA/I, and CS1 to CS6) as a model, the estimated vaccine coverage rate in children in Lima will be 92% (189/205). Further studies are needed to determine the utility of these antigens as well as other autotransporters in ETEC vaccines.

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VACCINATION FOR THE CONTROL OF TYPHOID FEVER: ESTIMATING THE POPULATION-LEVEL EFFECTS OF HISTORICAL TY21A FIELD TRIALS IN SANTIAGO, CHILE

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In the absence of water and sanitation interventions, vaccination remains a primary control measure for typhoid fever. Evaluating the herd protection of these vaccines is challenging, and is often compounded by changes in the environment. From 1982 to 1986, over 300,000 school children were vaccinated with at least one dose of Ty21a in the metropolitan region of Santiago, Chile. The incidence of typhoid fever declined in this area from over 100 per 100,000 at the beginning of this trial, to less than 50 per 100,000 at the end of 1990. Without a control population cluster, herd effects of the vaccine could not be directly estimated, or contrasted with the water and sanitation changes that occurred during this period. We use a mathematical modeling approach to estimate the vaccine's contribution to the decline in typhoid fever, both through the direct protection of the vaccine, as well as indirect protection through herd effects. Results from this study highlight methods for estimating the impact of typhoid vaccination in populations undergoing environmental change, as well as outline features for prospective typhoid vaccination trials important for the evaluation of both direct and indirect effects. These results can help inform strategies for global typhoid fever control, including the planning for new conjugate vaccine initiatives.

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ESCHERICHIA COLI PATHOTYPES FROM ECUADOR: ASSOCIATION WITH DIARRHEA AND ANTIBIOTIC RESISTANCE

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Diarrheagenic *Escherichia coli* (DEC) is an important cause of diarrhea in the developing world and the detection of these bacteria and their antibiotic resistance profiles are necessary for effective therapy. In this study, we conducted a microbiological survey of DEC in 233 stool samples, collected during a case control study in a hospital and health center in a low income neighborhood of Quito, Ecuador from April to September 2014. We used 8 sets of PCR primers to detect distinct DEC pathotypes. The overall prevalence of DEC was 30.5% in cases and 20.2% in controls (OR 1.76 CI 95% 0.96-3.20, p=0.06), Diffusely adherent E.coli (DAEC) was the most frequently detected pathotype in cases and controls (15.3% vs. 6.1% respectively) and was the only pathotype with a statistically significant association with diarrhea (OR 2.78, CI 95% 1.11-6.96, p=0.03). To our knowledge this is the first study investigating this pathotype in Ecuador. Additionally, pathotypes isolated from cases exhibited significantly higher levels of antimicrobial resistance to specific antibiotics, as well as higher levels of multidrug resistance, than isolates obtained controls.

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QUALITATIVE MOLECULAR DIAGNOSTICS MAY IMPROVE MEDICAL MANAGEMENT OF HOSPITALIZED SEVERELY MALNOURISHED CHILDREN WITH DIARRHEA: PRELIMINARY ANALYSIS FROM HÔPITAL DE L'AMITIÉ IN N'DJAMENA, CHAD

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Accurate diagnosis of infectious disease and rational use of antibiotics is challenging in resource-limited contexts where microbiological laboratory services are virtually absent. Diarrhea, for example, is typically present in at least 50% of children with severe acute malnutrition (SAM) admitted to hospital and is associated with increased risk of death. Current diarrheal treatment protocols for hospitalized SAM children rely on a syndromic approach, prioritizing fluid replacement and correction of malabsorption over specific treatment for pathogens. Antibiotic use is reserved for protracted or bloody diarrhea and is guided by a UNICEF protocol template applied without accounting for local or regional epidemiology. Studies suggest that multiplex molecular diagnostics can provide reliable clinically relevant information in field laboratories where infectious diseases like diarrhea represent a major portion of disease burden. Automated qualitative polymerase chain reaction (PCR) systems do not require a high level of expertise to operate and may provide actionable microbiologic information even though it gives no information regarding pathogen burden. ALIMA, with the support of the Institut Mérieux, is using qualitative PCR to test stools from hospitalized SAM children with diarrhoea in N'Djaména, Chad. Each sample is tested simultaneously for 22 distinct bacterial, protozoal and viral molecular targets. The trial expects 600 inclusions total over 12 months. Within the first 4 months, stool from 146 children have been analyzed. A total of 537 pathogens have been detected, an average of 3.8 per child. 70% are bacteria, 18% viruses and 12% parasites. Within the bacterial group 52% are E coli pathogens with entero-invasive E coli (EIEC)/Shigella detected in 57 of 146 samples (39%). Clinicians have adapted antibiotic prescription for 54/146 children (37%); 42/54 prescription changes result from the identification of EIEC/Shigella. Preliminary analysis of this trial demonstrates that the technology is accessible and reliable in a resource-limited context and that clinicians use the information to modify treatment.

1672

DRIED BLOOD SPOTS: AN ALTERNATE TOOL FOR THE ASSESSMENT OF IMMUNE RESPONSE TO CHOLERA INFECTION AND VACCINE

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Analyzing Dried Blood Spots (DBS) is an attractive tool for measuring antibody responses as it overcomes the challenges associated with supplies and expertise needed for venipuncture and sample processing, especially in resource-limited and challenging settings such as during cholera vaccine programs. The goal of this study was to evaluate DBS as a tool for the measurement of *Vibrio cholerae* O-specific polysaccharide (OSP)-antibody responses and to determine vibriocidal titers in volunteers immunized with oral cholera vaccine (OCV) Shanchol. Specifically, sera and blood spots were obtained from persons receiving OCV on day 0 (pre-vaccination), day 21 (21 days post 1st vaccine dose) and day 35 (14 days post 2nd vaccine

dose) in South Sudan. The results of assays involving DBS were correlated with traditional approaches utilizing sera. Blood spots were generated on Whatman 903 DBS cards, and stored at ambient temperature for up to 100 days. 4 dried blood soaked spots were punched out per volunteer specimen, and eluted overnight in a 24 well plate containing buffer. The eluates were subsequently used for determination of OSP-specific responses by ELISA. In a preliminary analysis involving 15 individuals, we noted statistically significant positive correlations between DBS and simultaneously sampled serum OSP-specific IgG (r=0.64, p<0.001), IgM (r=0.68, p<0.001) and IgA (r=0.64, p<0.001) antibody responses. Fold rises after the 1st OCV dose correlated between DBS and serum ELISA's for OSP IgG (r=0.61, p=0.01) and OSP IgA (r=0.76, p<0.001). Seroconversion rates assessed by the two methods were also similar. In addition, preliminary experiments suggest DBS can be used for the determination of vibriocidal titers using drop plate culture methods as determined by 50% growth reduction in samples compared to DBS free controls. Further assay optimization and validation is pending and more complete results will be available at time of presentation. Our data suggest a potential use of DBS as an inexpensive and convenient tool for the assessment of OCV immunogenicity and seroprevalence surveys, including in resource-limited settings.

1673

THE STUNTING SYNDROME DEVELOPS IN CHILDREN WITH INCREASED MICROBIAL TRANSLOCATION AND ATTENUATED EVOLUTION OF THE GUT MICROBIOME

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Stunting due to malnutrition is estimated to affect 165 million children under 5 years old. However, its pathophysiology remains elusive. We hypothesized that in low resource settings stunting is mediated by systemic inflammation due to increased microbial translocation ensuing from chronic intestinal damage and microbiome perturbation. We enrolled 81 healthy infants living in rural villages of Peru and followed them for 6 months with monthly growth assessments. Blood samples for markers of intestinal damage (intestinal fatty acid binding protein [I-FABP]) and systemic inflammation and stool samples for microbiome analysis were collected at months 0 (enrollment) and 6. Biomarkers were measured by Luminex or ELISA. Microbiome analysis was performed via 16SrDNA sequencing. Non parametric statistics were used to compare distribution of continuous variables and to measure correlations. Multivariate odds ratios of stunting were estimated by logistic regression. By 6 months, 18 (22%) children became stunted. I-FABP was high in cases and controls at month 0 and 6 but was not significantly different between the 2 groups at any time point. Tumor necrosis factor α, interleukin-1 and -6 levels were comparable between the 2 groups at month 0 and 6. Greater increases in soluble CD14 (monocyte activation) and lipopolysaccharide binding protein were associated with increased odds of stunting after adjusting for month 0 age and HAZ (ORs 7.24 ([95% CI 1.05- 49.84] P= 0.04) and ORs 6.32 ([95% CI 1.40-28.36] P=0.02 respectively). Children who became stunted had arrest of the physiologic increase in microbiome diversity over time and a different distribution of bacterial taxa compared to controls. Among Peruvian children younger than 2 years of age 1) markers of enterocyte damage are high 2) stunting is associated with increased microbial translocation/innate immune system activation and slower evolution of the gut microbiome. Interventions to prevent or repair intestinal damage may prevent the stunting syndrome.

1674

ANTIMICROBIAL RESISTANCE PROFILE IN ENTEROBACTERIAE ISOLATED FROM CHILDREN UNDER-2-YEARS-OLD IN PERI-URBAN COMMUNITIES IN LIMA, PERU

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Antimicrobial resistance is a major and growing problem worldwide, specially affecting low and middle-income countries. There is no recent data regarding antimicrobial resistance in enteric pathogens in children in Lima. The aim of this study was to determine the antimicrobial resistance patterns of important enteric bacteria isolated from stool samples of children under 2 years old. Stool cultures were obtained during a community trial in the District of Independencia, Lima, between 2008 and 2011. A total of 711 diarrheal samples and 348 controls (from asymptomatic children) were collected. *Shigella* and *Campylobacter* were identified by routine microbiology; diarrheagenic *E. coli* strains were indentified by real time-PCR. Antibiotic susceptibility was tested by disk diffusion. Diarrheagenic *E. coli* strains included 346 enteropathogenic (EPEC), 253 enteroaggregative (EAEC) and 177 enterotoxigenic *E. coli* (ETEC). Diarrheagenic *E. coli* pathotypes (n=776) were resistant to ampicillin (68%), trimethroprim-sulfamethoxazole (61%) and tetracycline (49%); with low resistance rates to ciprofloxacin and ceftriaxone (<4%). *Shigella* isolates (n=96) were resistant to ampicillin (65%), trimethroprim-sulfamethoxazole (83%), tetracycline (70%) and chloramphenicol (49%); resistance to ciprofloxacin and ceftriaxone was not found. *Campylobacter* isolates (n=187) were resistant to tetracycline (90%), ciprofloxacin (88%) and azithromycin (17%). There was no difference in the resistance rates between diarrheal and control samples. Antimicrobial resistance of enteric pathogens is high in this setting. There is an urgent need to implement intervention strategies to control the emergence and spread of resistant strains and large scale, prospective, multicenter surveillance studies to document the current trends.

1675

ENTEROAGGREGATIVE *ESCHERICHIA COLI* IS SYNERGISTIC WITH OTHER ENTERIC PATHOGENS TO IMPAIR GUT ABSORPTION, CAUSE INFLAMMATION AND IMPAIR GROWTH

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Enteroaggregative *Escherichia coli* (EAEC) is common in children in developing countries. We evaluated EAEC infections in monthly stools tested in 1226 asymptomatic children with over 90% of twice weekly follow up for their first 2 years of life across 8 MAL-ED sites in Asia, Africa and Latin America. When children with EAEC alone were compared with those with no pathogens, other pathogens, or EAEC with 1, 2 or 3 other pathogens, those with EAEC or any other pathogen had inadequate sanitation compared with those with no pathogen at any of the three periods. Poor sanitation, percent of mothers with <6 years of education, lower socioeconomic assessment or percent with income <$150/month were associated with EAEC coinfections compared to the other groups including those with pathogens other than EAEC. Antibiotic use and low percent of breastfeeding were also associated with EAEC coinfections. Myeloperoxidase was increased with EAEC coinfections compared also to all groups including the group with pathogens other than EAEC. Alpha-glycoprotein and neopterin were reduced with EAEC coinfection compared to all other groups. EAEC coinfections also showed gut dysfunction as measured by lactulose:mannitol absorption, driven mainly by decreased mannitol absorption, reflecting reduced mucosal absorptive area. EAEC

coinfections also associated with lower changes in weight-for-age, weight-for-length and length-for-age z scores compared with all other groups. The most frequent cumulative EAEC coinfections were *Campylobacter*, Giardia and atypical enteropathogenic *E. coli* (EPEC). We conclude that EAEC coinfections associate with lower socioeconomic status, sanitation, antibiotic use and breastfeeding. The most frequent EAEC coinfections were Campy, Giardia and aEPEC. EAEC interactions with pathogens also associate with intestinal inflammation decreased local and systemic immune responses and gut dysfunction especially reduced intestinal absorptive function. Thus subclinical EAEC infections appear to synergize with other pathogens more than they synergize without EAEC, to impaired growth in children across this multisite study.

1676

DOUBLE JEOPARDY: CHOLERA OUTBREAKS IN PRISONS IN THE 21ST CENTURY

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Cholera outbreaks in prisons have been described since the 1800s. In most countries, prisoners are among the most susceptible and neglected risk groups, and among the least likely to receive immediate care. To identify cholera outbreaks in prisons globally and the control measures used, we conducted a systematic search of medical journals and news sources for reports of cholera in prisons. Reports of outbreaks identified through personal communication with public health officials were also included. We identified 27 cholera outbreaks in 13 countries between 2000 and 2015 (18 in Africa, 8 in the Americas, and 1 in Asia). Five outbreaks were reported in medical journals, 16 by media sources, 4 in non-governmental organization blogs or reports, and 7 by other sources. The number of cases ranged from 5 to 450, with case fatality rates of 0%- 50%. Control measures included: isolation of ill prisoners; water, sanitation and hygiene interventions; prophylactic use of antibiotics; suspending visits and halting food deliveries; and setting up an emergency treatment center. Antibiotics reportedly controlled the spread of cholera in 3 of 4 outbreaks. Oral cholera vaccines were used in two outbreaks, in one as a direct control measure, and in another as a preemptive measure to prevent the spread of the outbreak to unaffected prisons. Vaccine impact was not assessed. Our search identified at least 27 cholera outbreaks that had been reported in prisons since 2000, sometimes resulting in high case counts and case fatality rates. Because of limited surveillance in prisons, reported outbreaks, cases and deaths are likely to considerably underestimate the scope of the problem. Though prisons can be a challenging setting, chemoprophylaxis or vaccination can be delivered quickly, effectively and at low cost, and can supplement other measures which often take longer to implement. Enhanced surveillance and a systematic approach to cholera prevention, preparedness, and response in prisons, as well as rigorous post-response evaluation, could demonstrate more precisely the impact of various combinations of interventions and inform future prevention strategies.

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DISTRIBUTION OF *E. COLI* PATHOTYPES ALONG AN URBAN RURAL GRADIENT IN ECUADOR

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Pathogenic *E. coli* is one of the primary causes of diarrhea in developing countries. We report on the results of the EcoZUR study (*E. coli* en Zonas Urbanas y Rurales - *E. coli* in Urban & Rural Areas), a case-control study of diarrhea at four sample collection sites in Ecuador, along a rural-urban gradient. We sampled ~100 subjects with diarrhea and ~100 age-matched controls without diarrhea at each of the four Ecuadorian Ministry of Health clinics, including a large clinic in Quito, Ecuador's capital (Pop. 1.62 million), a hospital in the capital of Esmeraldas Province (Pop. 162,000), the town of Borbón (Pop. ~5,000), and outlying rural communities in the Borbón region, along the Onzole, Cayapas, and Santiago Rivers (Pops. ranging from ~10-500). The urban-rural gradient also represents a gradient of access to clean and safe water. We cultured *E. coli* from the fecal samples and used a set of 8 PCR primers to test them for virulence factors associated with diarrheagenic *E. coli* pathotypes. We found very high rates of diffuse adherent *E. coli* (DAEC) in study subjects in Quito and Esmeraldas, with ~10% and ~20% of study subjects, respectively, positive for this pathogen. Atypical enteropathogenic *E. coli* (EPEC) was the second most common pathotype detected. DAEC was the only pathotype significantly associated with diarrheal disease, although this may have been a function of sample size constraints, as the numbers for the other pathotypes were limited. We also report on association between a) diarrhea and b) presence of pathogenic *E. coli* and risk factors such as water treatment, sanitation type, and recent travel. Our study allows us to understand how factors that differ along a rural-urban gradient—such as diet, access to clean water and sanitation facilities, and travel patterns—affect the set of enteric pathogens circulating within a population.

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EPIDEMIOLOGY AND RISK FACTORS FOR CRYPTOSPORIDIOSIS IN CHILDREN IN THE MAL-ED STUDY

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Cryptosporidium spp are enteric protozoa that cause significant morbidity and mortality in young children worldwide. The Etiology, Risk Factors, and Interactions of Enteric Infections and Malnutrition and the Consequences for Child Health and Development Project (MAL-ED), a cohort study across eight sites, allowed an ideal opportunity for defining the epidemiology of *Cryptosporidium spp* in children living in resource-limited settings. Children were enrolled within 17 days of birth. Data on illness, socioeconomic variables, and nutritional intake were collected by survey. Stool samples were tested for *Cryptosporidium parvum* by ELISA. Anthropometric measurements were taken monthly. The final analysis included 1,659 children with 24 months of follow up. Across the eight sites, 51.1% (848/1659) of children had at least one infection with *Cryptosporidium spp*, and 48.9% (811/1659) remained infection-free. *Cryptosporidium* diarrheal episodes were more likely to be associated with dehydration (16.5% vs 8.3%, $p < 0.01$), and to meet the GEMS definition of moderate-to-severe diarrhea (20.3% vs 11.9%, $p < 0.01$). Rates of *Cryptosporidium* diarrhea were highest in the Peru (10.9%) and Pakistan (9.2%) sites. Detection rates in surveillance stools ranged from 2.7% in Brazil to 6.6% in Tanzania and 7.3% in Peru. Children in rural sites had a significantly quicker progression to first *Cryptosporidium* infection than children from non-rural sites. Stunting at baseline was not associated with higher risk of infection (mean HAZ -0.92 vs -0.99, $p = 0.16$). Lower family income (146.94 (132.97) USD vs 199.22 (188.96) USD, $p <0.001$), overcrowding (29.6% vs 21.1%, $p<0.001$), fewer years of maternal education (6.25 (3.99) vs 7.35 (3.96), $p < 0.001$), and unimproved sanitation (chi squared 29.64, $p < 0.0001$) were all associated with *Cryptosporidium* infection. In this cross-site analysis, rural sites had greatest burden of disease, earliest onset of disease, and highest prevalence of unimproved sanitation, suggesting that interventions targeting spread of cryptosporidiosis should focus on improved sanitation infrastructure.

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THE SPECTRUM OF *CHROMOBACTERIUM VIOLACEUM* INFECTIONS FROM A SINGLE GEOGRAPHIC LOCATION

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Chromobacterium violaceum is a bacterium associated with soil and water exposure in tropical regions and causes rare and serious clinical infections that are often fatal. We reviewed the demographic and clinical details of twenty-eight patients with *C. violaceum* detected over fifteen years from 2000 to 2015, from the Top End of the Northern Territory. Eighteen patients had infections attributable to *C. violaceum*. Patients with infections were more commonly male (55.6%), and in the 16-to-60 year (61.1%) age group. Skin and soft tissue infections (50%), predominantly involving the limbs, were the major clinical manifestation. Water, mud exposure and trauma were all noted as precipitating circumstances and co-morbidities were present in 61.1% of the patients with infections. Ten of the twenty-eight patients (35.8%) had *C. violaceum* isolated as an incidental finding or as asymptomatic colonisation; these ten patients did not require, or receive therapy for the presence of *C. violaceum* bacteria. There were no relapsing infections in this group. *C. violaceum* remains a serious infection, with seven patients (25%) in our series requiring intensive care management. However, the mortality rate (7.1%) in our series was far lower than previously described. This case series of *C. violaceum* infections from a single geographic area provides additional information of the characteristics of infection with this pathogen.

1680

TARGET PRODUCT PROFILE FOR A DIAGNOSTIC ASSAY TO DIFFERENTIATE BETWEEN BACTERIAL AND NON-BACTERIAL INFECTIONS TO GUIDE ANTIMICROBIALS USE IN RESOURCE-LIMITED SETTINGS: AN EXPERT CONSENSUS

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Acute fever is one of the most common presenting symptoms globally. In order to reduce the empiric use of antimicrobial drugs and improve outcomes, it is essential to improve diagnostic capabilities. In the absence of microbiology facilities in low-income settings, an assay to distinguish bacterial from non-bacterial causes of fever would be a critical first step. To ensure that patient and market needs are met, the requirements of such a test should be specified in a target product profile (TPP). To identify minimal/optimal product characteristics for a bacterial vs. non-bacterial fever test, experts from academia and international organizations with expertise in infectious diseases, diagnostic test development, laboratory medicine, global health, and health economics were convened. TPP characteristics were proposed and reviewed by a working group, and consensus characteristics were defined. This working group defined non-severely ill, non-malaria infected children as the target population for the desired assay. To provide access to the most patients, the test should be deployable to community health centers and informal health settings, and staff should require <2 days of training to perform the assay. Further, given that the aim is to reduce inappropriate antimicrobial use as well as to deliver appropriate treatment for patients with bacterial infections, the working group agreed on minimal diagnostic performance requirements of >90% and >80% for sensitivity and specificity, respectively. Other key characteristics, to account for the challenging environment at which the test is targeted, included: i) time-to-result <10 min (but maximally not >2 hrs); ii) storage conditions at 0-40°C, ≤90% non-condensing humidity with a minimal shelf life of 12 months; iii) operational conditions of 5-40°C, ≤90% non-condensing humidity; and iv) minimal sample collection needs (50-100µL, capillary blood). This expert consensus approach to define assay requirements for a bacterial vs. non-bacterial diagnostic assay should guide product development, and enable targeted and timely efforts by industry partners and academic institutions.

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INVASIVE NON-TYPHOIDAL *SALMONELLA* INFECTIONS IN ASIA: CLINICAL OBSERVATIONS, DISEASE OUTCOME AND DOMINANT SEROVARS FROM A TERTIARY REFERRAL HOSPITAL IN HO CHI MINH CITY, VIETNAM

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Invasive non-typhoidal Salmonella (iNTS) infections are now a well-described cause of morbidity and mortality in children and HIV-infected adults in sub-Saharan Africa. In contrast, the epidemiology andclinical manifestations of iNTS disease in Asia are not well documented. We retrospectively identified >100 cases of iNTS infections in an infectious disease hospital in SouthernVietnam between 2008 and 2013. Clinical records were accessed to evaluate demographic and clinical factors associated with iNTS infection and identify risk factors associated with death. Multi-locus sequence typing and antimicrobial susceptibility testing was performed on all organisms. Of 102 iNTS patients, 71% were HIV-infected, >90% were adults, 71% were male and 33% reported intravenous drug use. Twenty-six/92 (28%) patients with a known outcome died; HIV infection was significantly associated with death (p=0.004). S. Enteritidis (ST11) (48%, 43/89) and S. Typhimurium(sequence types (STs)19, 34 and 1544) (26%, 23/89) were the most commonly identified serovars; S. Typhimurium was significantly more common in HIV-infected individuals (p=0.003). Isolates from HIVinfected patients were more likely to exhibit reduced susceptibility against trimethoprim-sulfamethoxazole than HIV-infected patients (p=0.037). We conclude that iNTS disease is a severe infection in Vietnam with a mortality rate similar to sub-Saharan Africa. As in sub-Saharan Africa, HIV infection is the major risk for death, with the majority ofthe burden in this population in HIV-infected men. Although the STs of iNTS organisms identified in this study were common globally, we suggest continued surveillance across Asia to monitor for the presence of multi-drug resistant STs.

1682

COXIELLA BURNETII ANTIBODIES ARE PREDOMINANT AMONG PATIENTS WITH UNDIFFERENTIATED FEVER IN AFGHANISTAN

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Diagnosis of infectious diseases in Afghanistan remains a challenge with limited ability for pathogen isolation and identification. Baseline data on the prevalence of etiologies causing undifferentiated fever is lacking in Afghanistan. Herein we screened serum of Afghan patients suffering from undifferentiated fever (UF) for antibodies against number of pathogens, including *Coxiella burnetii, Leptospira* spp.and typhoid fever. Patients > 5 years old with UF who meet the WHO case definition and presented at

two provincial hospitals: Kandahar (KDH, n=178), Helmand (LG, n=82) and a third quaternary level hospital in Kabul (KID, n=303) were enrolled and consented into a surveillance study between 2007 and 2012. A single serum sample was collected and tested by ELISA for the detection of IgM and IgG against Q fever (*C. burnetii), Leptospia* spp. IgM and total immunoglobulins of *Salmonella enterica* serovar typhi. A total of 563 patients were screened and 50.3 % were seropositive against at least one pathogen. Cases from KDH showed the highest frequency of *C. burnetii* antibodies (n=80, 37 % IgG and 8.4 % IgM), followed by those from LG (n= 21, 20.7% IgG and 4.9% IgM) and KID (n= 55, 16.5% IgG and 1.7% IgM). Leptospia IgM was evident in 11.4% of patients, 13.2% in KID, 10.7 in KDH and 6.1 in LG. Typhoid fever titers >320 were found in11.2% of all patients, being higher in LG (15.9%) and KDH (12.9%) than KID (8.9%). Almost half of the *C. burnetii* IgM-positive cases (12/22) did not mount immune responses to other pathogens. The data suggest that both acute and past Q fever infections were evident within patients tested. The increased seropositivity rates in cases from KDH and LG provincial hospitals compared to those of KID in Kabul city may be attributed to limited sanitary measures for typhoid fever. While typhoid fever is transmitted via ingestion of polluted food and water, both Q fever and Leptospira are spread by contact with animals, their contaminated products or excreta. The current results provide initial disease burden data for Afghanistan and will be useful to health authorities in guiding hygiene improvement plans and disease prevention strategies.

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DETECTION OF *SALMONELLA BACTEREMIA* IN RURAL KENYA USING FIELDABLE DIAGNOSTICS

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Invasive Non-Typhi Salmonella (NTS) are a major cause of infections in sub-Saharan Africa, with a case fatality rate of 4-27% in children and 22-47% in adults. The extensive presence of other co-morbidities in the patients complicates diagnostics. There is a need for rapid, reliable, and fieldable diagnostics that can be used at the point-of-care. We are developing such diagnostic assays for Salmonella detection using two approaches - pathogen biomarker detection using a waveguide biosensor, and real-time PCR. The former requires minimal handling of samples, and allows for the rapid and specific detection of antigen using fluorescent probes and uses a novel assay strategy called lipoprotein capture. Salmonella lipidic biomarkers are taken up by lipoproteins in serum, which are immobilized on sensor surface, and the associated biomarker is identified using Salmonella-specific antibodies. The assay was optimized using lipid lysates prepared from either control strains of Salmonella or clinical strains from patients in Kenya. The optimized assay was tested on four pediatric samples from Kenya. We saw excellent (100%) corroboration with culture results for the samples. Future work includes identification and characterization of the antigen, and testing more patient samples from Kenya. Real-time PCR requires more handling of samples and expertise to run the assays but are useful for the characterization of isolates and identification of resistance. For PCR assays, primers for Salmonella detection were designed and tested with DNA from control and clinical isolates. Testing is underway with a Gram detection assay to differentiate between Gram-positive and -negative bacteremia. We are also working on identifying antimicrobial resistances in the isolates from Kenya to design assays for their detection, and have identified resistance to first, second and third-line antibiotics in patients. Further validation for the PCR assays will be done on clinical samples. Our goal is to deploy these technologies to our clinical site in rural Kenya, and train local personnel to run them, thereby improving health care infrastructure in country.

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LEPTOSPIRAL DNA IN FEBRILE PATIENTS FROM SEMI-RURAL COMMUNITIES IN MANABÍ-ECUADOR

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The aim of this study was determine whether fever is associated with the presence of leptospiral DNA in human sera detected with Polymerase Chain Reaction (PCR). DNA was extracted from 576 samples of human serum (513 febrile and 63 non-febrile) obtained between February 2014 to July 2015 from semi-urban parishes Calderón and Santa Ana in Portoviejo city. DNA was analyzed first with real time PCR (PCR-RT), followed by conventional PCR and finally amplicon sequencing. The 16s ribosomal RNA sequences were detected in 2 out of 513 (0.5%) febrile patients and 0 of 63 (0.0%) from non-febrile patients.

1685

PROGRESS AND CHALLENGES OF TRACHOMA ELIMINATION IN THE FAR NORTH REGION OF CAMEROON

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In Cameroon, trachoma mapping conducted in 2010-2011 identified 13 health districts (HDs) in the Far North region with a prevalence of trachomatous inflammation-follicular (TF) of over 10% in children aged 1-9 years. These HDs qualified for district mass drug administration (MDA) as well as implementation of other components of the SAFE (Surgery, Antibiotic treatment, Facial cleanliness and Environmental improvement) strategy recommended by World Health Organization. These HDs benefitted from support from HKI with funding from USAID's ENVISION Project, managed by RTI International. Of these 13 HDs, 5 successfully passed impact assessment in 2014 and stopped MDA. In 2015, 5 additional HDs completed 3 rounds of MDA with coverage of >80% and qualified for impact assessment. However, due to continued insecurity caused by Boko Haram attacks, only 2 HDs (Mokolo, Guidiguis) were evaluated. A cross-sectional, cluster randomized survey was conducted to estimate the TF prevalence to determine if the stopping MDA criteria had been met. A sample of 1,961 children aged 1-9 years was surveyed. The WHO simplified trachoma grading system was used. The results showed that the TF prevalence decreased from 16.9% (95% CI: 15.4-18.5%) and 13.1% (95% CI: 11.7-14.5%) in 2010 to 1.0% (95% CI: 0.5-1.8%) and 0.8% (95% CI: 0.3-1.7%) in 2015 in Guidiguis and Mokolo respectively. These 2 HDs reached a TF prevalence of <5% and hence met the criteria of stopping MDA. While these results represent further positive steps towards trachoma elimination in the region, the threat of terrorism in these regions has formed a barrier to timely completion of surveys and hence measuring the progress of the program. Uncertainty remains for the 3 HDs not yet assessed, and according to the plan, 2 more HDs are scheduled for evaluation in 2016 and 1 additional HD in 2017. It is not clear whether these surveys can go ahead as planned. In addition, insecurity makes it difficult to evaluate the progress of the other SAFE components such as TT surgery and environmental improvements. These challenges are a serious threat to achieving the year 2020 trachoma elimination goals in Cameroon.

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THE BURDEN OF TRACHOMA IN EASTERN EQUATORIA STATE, REPUBLIC OF SOUTH SUDAN

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Eleven counties in the Republic of South Sudan required trachoma impact surveys in order to evaluate the success of program interventions and to determine if additional rounds of mass drug administration (MDA) with azithromycin were needed. Despite security issues and population displacement The Carter Center supported the South Sudan Ministry of Health (MoH) to conduct surveys in five counties in Eastern Equatoria state. These were the first known trachoma impact surveys since the country's independence and the first population-based clinical trachoma data to be collected in this region since 2006. Owing to limited in-country personnel capable of serving as trachoma grader trainers, the MoH reached out to neighboring countries Ethiopia, Sudan and Uganda who provided experienced grader trainers for the training. For each survey, a multi-stage cluster-random sampling method was used. Households within a cluster were selected with equal probability and all present household members were examined using the WHO simplified trachoma grading scheme for all 5 clinical signs of trachoma. Despite remoteness of villages, difficult terrain and weather conditions, a total of 14,462 individuals in 3,446 households were surveyed across the five counties in Eastern Equatoria state. The burden of trachoma was high in these 5 counties. The prevalence of trachomatous inflammation follicular (TF) in children age 1-9 years ranged from 21.1%, (95% Confidence Interval (CI):12.1, 34.1) to 47.6%, (95%CI: 42.2, 53.1). Trachomatous trichiasis was also highly prevalent, ranging between 2.9% to 4.5% in those 15 years and older. The prevalence of water and sanitation indicators were low in all five counties, including two counties which had a complete absence of latrines in all surveyed villages. The results of the survey showed that all 5 counties will require at least 3 to 5 more years of MDA and surgical interventions. This experience also shows that surveys can still be carried out in extremely resource poor and difficult areas, and that neighboring countries are willing to provide valuable technical assistance.

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IMPACT OF MASS TREATMENT WITH AZITHROMYCIN FOR TRACHOMA ON SEXUALLY TRANSMITTED INFECTIONS AND ANTIMICROBIAL RESISTANCE AMONGST WOMEN IN THE SOLOMON ISLANDS

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Chlamydia trachomatis is the most common bacterial sexually transmitted infection and is frequently asymptomatic. Population based interventions aiming to increase the coverage of screening for C. trachomatis and behavioural interventions to reduce high-risk sexual behaviour have shown limited efficacy in reducing its prevalence. Resistance to first line antimicrobials is an increasingly significant problem in the management of sexually transmitted infections and threatens the effectiveness of current treatment regimes. Ocular infection with C. trachomatis is the cause of trachoma, which is also endemic in the Pacific. The WHO strategy for the elimination of trachoma as a public health problem includes community mass treatment with azithromycin. Mass drug administration (MDA) of azithromycin for trachoma might reduce the prevalence of genital C. trachomatis but might also drive the emergence of antimicrobial resistance. We conducted a study in the Solomon Islands alongside a Ministry of Health trachoma elimination programme to establish the impact of MDA with azithromycin on sexually transmitted infections and look for evidence of emerging drug resistance. Women attending outpatient clinics before or after MDA were enrolled. Self-taken high vaginal swabs were tested by PCR for C. trachomatis and Neisseria gonorrhoeae. Whole genome sequencing was attempted on all samples that were positive by diagnostic PCR for either pathogen. Following MDA, we noted a significant decrease in the prevalence of genital C. trachomatis infection, but not of N. gonorrhoeae, by diagnostic PCR. We will present data from whole genome sequencing of genomic evidence of the presence/absence of antimicrobial resistance obtained from both the pre and post-MDA samples.

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QUALITY ASSESSMENT OF THE IMPLEMENTATION OF THE TRACHOMATOUS TRICHIASIS SURGERY IN POLI HEALTH DISTRICT, CAMEROON USING SWPO METHOD (SUCCESS - WEAKNESSES - POTENTIALS - OBSTACLES)

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Poli is a trachoma endemic district in the North Region of Cameroon, with a total population of 88,513. The TT prevalence was 1.08% at baseline mapping. As standardized by sex and age, the estimated backlog is 625 cases, corresponding to an Ultimate Intervention Goal (UIG) of 536. A TT surgery campaign was organized in February 2016, supported by the United States Agency for International Development's MMDP Project, managed by Helen Keller International. This was preceded by a series of training of trainers, surgeons and nurses using the HEAD START mannequin, supply of drugs and consumables, community meetings, and dissemination of awareness messages. During the campaign, 3203 patients were examined and 92 TT cases were managed, including 84 cases operated and 8 cases that refused the surgery, but received tweezers and advices for adequate epilation. With the aim to appreciate the quality of the implementation of the campaign, a self-assessment using SWPO method (Success - Weaknesses - Potentials - Obstacles) was carried out by stakeholders at different levels of the health pyramid. This consisted of reviews of all steps of implementation of each activity conducted, to identify strengths, weaknesses, obstacles and potentials that could be explored and taken into consideration for the planning of upcoming campaigns. The community meetings held in all villages were identified as a success that allowed the dissemination of sensitization messages. Challenges with timing of the surgeon training, the availability of cases for the surgeon training, and the procurement of surgical supplies constituted the main weaknesses. The spatial distribution of TT cases in the district does not reflect the forecasts resulting from baseline data, and this constituted a significant obstacle. There is a potential to use community meetings for preliminary screening of suspected TT cases before deploying surgical teams to the field. Experiences and opinions of stakeholders involved in the TT surgery campaign in Poli allowed us to collect information that will be taken into account in planning next TT surgery campaign in the North region.

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FACTORS PREDICTING TRACHOMA IMPACT SURVEY OUTCOMES

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Trachoma-endemic countries are committed to achieving the elimination of trachoma as a public health problem by 2020. To do this, multiple years of the WHO-endorsed SAFE strategy are recommended, depending on baseline prevalence of trachomatous inflammation-follicular (TF) and trachomatous trichiasis (TT). Trachoma impact surveys (TIS) have been implemented in 9 countries supported by USAID between 2012-2015 to determine whether TF and TT prevalence have decreased to a point where further intervention is no longer required. We measured the effects of baseline TF prevalence, number of rounds of MDA, and median MDA coverage on the likelihood of passing a TIS. Results from one hundred forty-three TIS conducted between 2012 and 2015 across 9 countries were analyzed using logistical regression analysis. Out of 102 surveys implemented in 8 countries, 83% showed that the TIS passed (range 60-100%). Preliminary logistic regression analysis of baseline TF prevalence, number of MDA rounds and median coverage variables showed that baseline TF prevalence was statistically associated with passing TIS (alpha=0.05): odds ratio =0.934; 95% confidence interval 0.898-0.970. However, when the results from 70 TIS implemented in one highly endemic country—all but 2 of which failed—are included in the analysis, the model no longer holds. Median baseline TF prevalence in these 70 districts was 39%, compared to 11.8% in the other 102 districts, and the median number of MDA rounds was 6 compared to 3. Median coverage in this country was high, at 94.5%, compared to 82.9% in the other 8 countries. Districts with low baseline prevalence were significantly more likely to pass trachoma impact surveys in 8 countries; for every percentage point increase in baseline prevalence, the odds of passing TIS decrease by 6.6%. Despite high MDA coverage over multiple rounds in one country, the TIS did not pass. This analysis confirms that especially in areas of very high baseline prevalence, high coverage of antibiotics alone is not sufficient for decreasing TF prevalence; F&E interventions should also be prioritized for sustainable elimination of blinding trachoma to be achieved.

1690

CAN WE ELIMINATE TRACHOMA AS A PUBLIC HEALTH PROBLEM BY 2020?

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Trachoma remains the world's leading infectious cause of blindness. The World Health Organization (WHO) is aiming to eliminate trachoma as a public health problem by 2020. In order to achieve this they have outlined the ultimate intervention goals, the first of which aims to achieve a reduction in Trachomatous Inflammation - Follicular (TF) to less than 5% in children 1-9 years old within all endemic communities by 2020. We use data collected by the International Trachoma Initiative on the prevalence of TF in 44 trachoma endemic districts to assess the feasibility of achieving this goal by 2020, using a statistically validated dynamic mathematical transmission model. For each endemic district that had not reached the current target by the most recent time point of surveillance, we assess whether the ultimate intervention goal will be met by 2020 using the current WHO guidelines alone and, if not, what additional interventions may be required. We find that for regions with greater than 30% TF prevalence, current mass drug administration (MDA) regimes are not sufficient to achieve this goal. Our work suggests that an increased frequency of MDA treatment, in addition to enhanced facial cleanliness and environmental improvements (resulting in long-term transmission reduction) will be essential in these regions. Moreover, in regions where TF is less than 30% at least some degree of long-term transmission reduction, through enhanced facial cleanliness and environmental improvements, is required in order to prevent re-emergence in the community in the absence of sustained and on-going MDA. Our findings suggest that in most districts where TF is less than 30% the first ultimate intervention goal is achievable with the current recommended WHO guidelines. However, considerably more resources will be required in high prevalence settings in order to ensure full elimination as a public health problem and elimination timelines are likely to exceed past 2020 in these settings.

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TRAINING OF MASS DRUG ADMINISTRATION (MDA) DISTRIBUTORS, COMMUNITY MOBILIZATION AND COMMUNITY KNOWLEDGE OF MDA: A QUALITATIVE POST-MDA ASSESSMENT AMHARA, ETHIOPIA

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In communities where the prevalence of trachoma exceeds a predetermined threshold annual community-wide mass drug administration (MDA) of antibiotics is warranted. Training of MDA distributors, community mobilization and knowledge of MDA are instrumental to effectively implement MDA. Despite this, most coverage surveys only focus on self-reported medication use by beneficiaries while ignoring the methodologies and structures through which MDA is implemented. To evaluate the training of MDA distributors, assess the role of health workers in mobilizing the community, and to assess the level of community awareness about the MDA campaign, a qualitative post-MDA assessment was conducted in Amhara, Ethiopia, an area hyperendemic for trachoma. Following an antibiotic MDA in 2015, four districts were selected for the administration of key stakeholder interviews with health workers at various levels of the health care system and focus group discussions (FGDs) with community members within randomly selected villages. A total of 12 interviews and 4 FGDs were conducted which included a total of 35 participants and results were recorded, transcribed, and analyzed using MaxQDA qualitative software to identify the primary themes described by participants. The themes included key stakeholder engagement, training, and community mobilization. More specifically the results showed multi-sector involvement of stakeholders during the MDA was thought to be very important and although training of drug distributors was consistently implemented in accordance with standardized procedures, respondents believed more time was needed for the training. With respect to community mobilization, health education was described as "essential" to ensure participation, and a greater involvement of community-level health volunteers would help governmental health workers increase mobilization and MDA coverage at the village level. The results from this study can be used to improve MDA distribution throughout the Amhara region, and will be instructive in other regions of Ethiopia currently scaling up trachoma MDA.

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MORTALITY FOLLOWING DISCHARGE IN CHILDREN ADMITTED TO A RURAL MOZAMBICAN HOSPITAL: DEVELOPMENT OF A PREDICTION MODEL TO IDENTIFY CHILDREN AT RISK OF DEATH

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An important but neglected contributor to child mortality is the vulnerable period following hospital discharge. The objective of this study is to determine the burden of post-discharge mortality and what are the predictors of mortality following discharge in a rural Mozambican hospital. A systematic review of the paediatric deaths taking place at community level over the last 11.5 years was done through a demographic surveillance ongoing in a southern district of rural Mozambique. We used a morbidity surveillance system ongoing in Manhiça District Hospital to exclude hospital deaths. We determined post-discharge mortality over three different time-periods: 1st: 0-30 days, 2nd: 31-60 days and 3rd:61-90 days following hospital discharge. We identified predictors of post-discharge mortality and derived a simple prediction tool that uses some of the collected variables to identify children at high risk of death after discharge. Data from 21227 children were reviewed and analysed (initial sample were 45700 children, of which 23725 lived out of study area, 555 were hospital deaths and 55 had missing outcome and were excluded). Mortality at 1st period was 2.3% (484/21227), at 2nd period was 1.4% (289/21227) and 3rd period was 1.0% (212/21227). Overall mortality was 4.6% (985/21227). The final adjusted model for the prediction of post-discharge mortality included the variables non breastfeeding among children <2 years (OR 2.4, 95%CI 1.7-3.1), orphan of both parents (OR 9.2, 95%CI 5.4-15.9), severe dehydration (OR 3.3, 95% CI 2.2-5.2), oral candidiasis (OR 8.1, 95% CI 6.1-10.7), Blantyre Coma Scale score <2 (OR 3.2, 95% CI 1.0-10.5), HIV-positive status (OR 12.1, 95% CI 5.1-28.4) and being <2years old (p<0.001). Mortality following discharge is a poorly recognised contributor to child mortality. A simple prediction tool that uses several easily collected variables can be used to identify children at high risk of death after discharge.

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LYMPHATIC FILARIASIS TRANSMISSION INTENSITY ASSESSMENT IN THE URBAN AREA OF BAMAKO, MALI

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Anopheles gambiae and *An. funestus* are the main vectors of LF in Mali. *Culex* spp is the most common mosquito species in urban areas of this region. The 2004 mapping survey using ICT found that all regions in Mali were endemic for lymphatic filariasis (LF). We aimed to determine if *Culex* species are incriminated in *Wuchereria bancrofti* (Wb) transmission in the capital city, Bamako, and to assess the endemicity level in Bamako after 3 mass drug administration (MDA). Vectors were trapped using CDC light traps and tested for Wb infection by PCR. Night thick smears were collected from volunteers >14 years in the 6 national LF elimination program sentinel sites in Bamako and in two additional urban sites selected because of a family with 3 cases of elephantiasis (Faladie) and the high frequency of *Culex* breeding sites (Bozola). A total of 6,174 *Culex* spp (85.2%), 16 *An. gambiae* (0.2%), 26 *Aedes* spp (0.4%), 858 *Ceratopogonidea* (11.8%) were collected. No positive *Culex* pools were detected among the 252 tested by PCR. None of the 1,002 volunteers had detectable Wb microfilariae. Mp microfilariae were detected in 5 individuals in two of the localities (0.5%). These data provide no evidence of active LF transmission requiring intervention in Bamako. The presence of Mansonella perstans microfilaremia in residents of two of the eight localities is consistent with migration from rural to urban areas in this population.

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SCRUB TYPHUS AS A MAJOR CAUSE OF ILLNESS FOR PATIENTS WITH UNKNOWN FEVER ORIGIN IN GALLE, SRI LANKA

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Scrub typhus (ST) is an acute febrile illness that is caused by the gram negative intracellular bacteria, *Orientia tsutsugamushi.* Traditionally, the diagnosis of ST mainly relies on serologic tests. IFA is considered the gold standard for cases of seroconversion or a >4-fold rise in antibody titers between acute- and convalescent-phase serum specimens. ST is under-reported due to the nonspecific symptoms and the lack of simple diagnostic tests. Recent studies of acute febrile illness (AFI) in Southern Sri Lanka suggest a broad spectrum of infectious agents including *Orientia*. Here we used the 3-patented-recombinant-protein ELISA for the detection of *Orientia*-specific antibodies in AFI patients from Sri Lanka. Among all 460 pairs of serum tested, 80 of them were positive for IgG in the convalescent sera (17.4%). The acute sera of these 80 patients were further analyzed. Nine of them appeared to have IgG serocoversion, 40 of them had positive IgM for acute sera and 3 of them were both IgG seroconverted and acute IgM positive. Taken together, 58 (12.6%) patients were considered ST positive. Additional assays for the detection of other disease-specific antibodies of the remaining 85% patients without a definitive diagnosis are needed.

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LEPTOSPIROSIS IS ONE OF THE MAJOR DISEASES FOR PATIENTS WITH UNKNOWN FEVER ORIGIN IN GALLE, SRI LANKA

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Leptospirosis is caused by spirochaetes of the genus *Leptospira*. It is considered to be the most widespread bacterial zoonotic disease in the world. Timely diagnosis is essential for antibiotic therapy provides the greatest benefit when initiated early in the course of illness. Currently,

MAT is the standard serological method for the diagnosis of leptospirosis. However, it is technically complex and time-consuming. We have developed a 4-recombinant protein-based ELISA to detect *Leptospira*-specific antibodies. A total of 460 pairs of serum collected from febrile patients was anlayized. Among all 460 paired serum, 148 of them were positive IgG in the convalescent sera (32.1%). The acute sera of these 148 patients were further analyzed. Among these patients, 73 of them appeared to have IgG serocoversion between acute and convalescent sera, 16 of them had positive IgM for acute sera and 11 of them were both IgG seroconverted and acute IgM positive. Taken together, 82 (21.7%) patients were considered leptospirosis positive. While these results suggest that leptospirosis is a significant cause of illness with unknown fever origin in this cohort, there are still high percentage of patients without a definitive diagnosis.

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ANTIVENOM INDUCED ANAPHYLAXIS FOR TREATING NEUROTOXIC SNAKE ENVENOMATION IN NEPAL

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Intravenously (IV) administered snake antivenom is a life-saving treatment for snakebite envenomation but adverse reactions are common, including life-threatening, antivenom related anaphylaxis (ARA). Herein, we report our experience of ARA when treating neurotoxic envenomation caused mostly by Naja naja (Indian cobra) and Bungarus caeruleus (Indian krait) in Nepal. We conducted a double blind, randomised, trial of lower vs. higher dose of antivenom (IV push followed by an infusion). Patient monitoring involved symptoms, vital and neurotoxic signs and oximeter measured oxygen saturation. Prestudy training emphasised immediate ARA treatment when first recognised: stopping antivenom, administering intramuscular (IM) adrenaline, IV hydrocortisone and IV chlorphenamine, and salbutamol nebulisers, supplemental oxygen, intubation and hospital transfer, as clinically indicated. Results From April 2011 to November 2012, 154 envenomed patients were recruited of whom 13 [8.4 (95% CI: 4.6-14.0)]% had clinical features consistent with ARA: 3 children (5, 6, 11y) and 10 adults (18-52y). Nine had clear cut ARA whilst in four differentiation from envenomation was difficult. Median (range) time to first ARA was 5 (19-115) minutes. Typical ARA features included urticaria/erythema (n=10), hypotension/shock (5), dyspnea with (3) or without (4) wheezing. Unusual features were laryngeal oedema (2) and bradycardia (4). Sudden deterioration with cardio-and/or respiratory arrest occurred in 5 patients. Eight patients died, five within 4h and three at 13h (late laryngeal oedema, 24h (cardiac arrest while ventilated) and 11 days (ventilator associated pneumonia). Three deaths occurred in transit to hospital. In conclusion, ARA was relatively common and associated with a poor prognosis in resource restrained Nepal. More training, better anaphylaxis management and quality antivenom may save more lives.

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LUNG NODULES IN CHRONIC SCHISTOSOMIASIS: A RARE CONDITION?

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Schistosomiasis is a neglected tropical diseases caused by the fluke worms of the genus Schistosoma. The infection can cause damage to the liver and to the genitourinary tract, depending on the species involved. The lungs can be affected both in the acute presentation of the infection (Katayama fever), and in the chronic phase. In the latter case, the damage is usually described as pulmonary hypertension; nodular patterns have been described rarely.Between May 2014 and October 2015 six immigrants of African origin were diagnosed at the Centre for Tropical Diseases (CTD) of Negrar (Verona), Italy, with lung nodules due to chronic schistosomiasis. A further case was found at the Infectious Diseases Clinic of Udine, Italy. The patients were screened for parasitic infections because of eosinophilia. An ELISA assay for *Schistosoma mansoni* resulted positive for all of them, and all but one had eggs (of *S. mansoni* and/or *S. haematobium*) in stool and/or urine. Four patients complained of respiratory symptoms (cough, chest pain), while the others underwent a routine, screening chest x ray. The radiological investigations (x ray and then CT scan) demonstrated, in all patients, multiple pulmonary nodules. The first five patients underwent a biopsy of the nodules (TB was the main suspected cause), and the histological examination revealed schistosome eggs. The last two patients had a presumptive diagnosis. All patients were treated with praziquantel 40 mg/Kg/day for three days, obtaining complete resolution of the radiological picture. In the same period, at the CTD, 120 cases of schistosomiasis were diagnosed; hence, the pulmonary nodular presentation represented 5% of all cases of Schistosoma infection in the study period. In conclusion, schistosomiasis should be in the differential diagnosis of pulmonary nodules in patients coming from endemic areas. A specific screening is recommended and, if the index of suspicion for other severe conditions is reasonably low, invasive procedures can be avoided or postponed. Treatment with praziquantel seems to be efficacy, and the response can be monitored with CT scan.

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WEIGHT-FOR-AGE GROWTH-RATE FAILURE IN INFANTS IS ASSOCIATED WITH AN ALTERED BLOOD GENE EXPRESSION PROFILE INDICATING REDUCED IMMUNE RESPONSIVENESS

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Undernutrition is associated with an increased risk of morbidity and mortality due to infectious disease. In addition to mucosal barrier dysfunction, a systemic immunodeficiency is likely at play; however, the molecular basis of immune dysfunction in undernutrition is largely unknown. Here using weight-for age Z-score (WAZ) growth trajectories in the Bangladeshi PROVIDE birth cohort, we analyzed the cumulative

effect of growth rate failure (GRF) over the first year of life on the baseline peripheral blood gene expression profile at one year of age. GRF was defined as a WAZ curve that deflected downward across at least two Z-lines without recovery during the 53-week observation period. RNA from whole blood was extracted and cDNA target preparation was optimized to avoid globin or ribosomal RNA transcripts. Using microarray transcriptomic analysis, we found 146 genes that were differentially regulated ($P <$ 0.05 and at least 2-fold absolute change) between the age-and gender-matched control (n = 10) and GRF (n = 10) groups. Bioinformatics analysis indicated involvement of these genes in diverse cellular processes including metabolism, regulation of growth and apoptosis, and response to viral infection. These results suggest that a GRF trajectory may be associated with an altered baseline blood gene expression profile skewed towards reduced immune responsiveness. Further pathway analysis with additional specimens will inform novel *in vitro* experimental conditions for mechanistic studies.

1699

MATERNAL IRON DEFICIENCY ANEMIA, MALARIA AND SOIL TRANSMITTED HELMINTHS ARE A MAJOR RISK FACTOR FOR ANEMIA IN EARLY CHILDHOOD

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Iron deficiency is common in pregnant women and young children worldwide and can lead to clinically significant anemia and impaired neurological development in children. We measured hemoglobin (Hb) levels in parallel birth cohorts (measured monthly then every three months from birth to age 3 years) of Kenyan children living in rural (N=246) and nearby urban areas (N=353). Mean Hb levels were identical in the two cohorts at birth, then diverged by an average of 2.6 gm/dL lower Hb levels in rural cohort from 6 to 18 months of age (P<0.0001), but converged by 21-24 months reflecting similar diets in the two cohorts. Malaria and soil transmitted helminth infections (STH) infections were low in both cohorts and did not differ. Since erythropoiesis in infancy depends heavily on iron stores acquired prenatally we hypothesized differences in Hb levels between the two cohorts arose from impaired maternal transplacental transfer of iron to the fetus. We examined the impact of maternal risk factors on Hb levels in their offspring between ages 6 through 18 months using a generalized linear model. Maternal risk factors included infections (malaria, HIV, schistosomiasis, filariasis, STH), Hb, parity, age, and markers of anemia (serum ferritin, soluble transferrin receptor [sTfR], C-reactive proteins). Three maternal risk factors independently accounted for the majority of reduced Hb levels in their offspring; i) sTfR >8ug/ml (-0.73 gm/dL reduction in Hb levels, p=0.04), ii) malaria (-0.80 gm/dL Hb reduction, p=0.02), and iii) STH (-0.79 gm/dL Hb reduction, p=0.03). The other maternal risk factors were not significantly associated with low in Hb levels in their children. Thus iron deficiency anemia arising from malaria and/or STH in mothers' results in reduced iron stores in their offspring and significant anemia. Improved iron supplementation and prevention of malaria and STH infection in pregnant women could have a profound impact on their child's health.

1700

MAPPING AND MANAGEMENT OF A LARGE SCALE DROUGHT-ASSOCIATED SCABIES OUTBREAK IN ETHIOPIA

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The impact of the severe drought in Ethiopia, attributed to El Niño weather conditions, has led to increase in the potential for outbreaks of communicable diseases. In September 2015, reports from drought affected regions indicated that drought affected areas were experiencing scabies on a very large scale. Given the likelihood that living conditions to be compromised by the drought, and reports of major social and personal impacts of scabies, we undertook a comprehensive assessment of scabies prevalence to plan interventions. Training was given to the health extension workers (HEW), who provide front-line community health services at the subdistrict level. A resource containing diagnosis and management guidelines was developed and distributed. The HEW were asked to conduct a house to house survey, and collect data using a simplified tool on the prevalence of scabies. Specificity of scabies diagnoses made by the health extension workers has been assessed. Ivermectin based treatment management has been done. A total of 450 HEW were trained in the 3 zones, and undertook scabies screening in a population of 1,125,770 across 68 districts. The prevalence of scabies across districts ranged from 0.2 to 60.7%., with 379,000 overall confirmed cases for a total prevalence of 33.7%. In the specificity assessment, the diagnosis of scabies made by the HEW in 251 cases was reviewed and 248 confirmed to have scabies (98.8%). The mean reported duration of illness was 5 months. Severe scabies was found in 42% of those with scabies, and 75.1% of cases had another family member scabies. Of all scabies cases, 39%were school aged children and 30 % of affected children had bacterial super infection. 11% of the students with scabies had dropped out from school because of scabies or/and drought, and 85% of those who dropped out had bacterial super infection. Treatment has been given to 800,000 patient and contacts. In conclusion, the scabies burden in the region is enormous, and complicated by the nutritional shortage emergency and water scarcity. A coordinated response is urgently needed to control the epidemic.

1701

PROGNOSTIC CLINICAL INDICATORS FOR FATAL DENGUE IN TWO ENDEMIC AREAS OF COLOMBIA: A HOSPITAL-BASED CASE CONTROL STUDY

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The WHO estimates that about 98% of dengue fatal cases could be prevented; however, countries such as Colombia have recorded higher rates during recent epidemics. Our aim was to identify predictors of mortality that allow risk stratification and timely intervention of dengue patients. We conducted a hospital-based, case-control (1:2) study in 2 endemic areas of Colombia (2009-2015). Fatal cases were defined as having one the following: 1) positive serological test (IgM); 2) positive

virological test (NS1 or RT-PCR or viral isolation); 3) autopsy findings (macro and microscopic) compatible with death from dengue. Controls were inpatients with a positive serological or virological dengue test (frequency matching by state and year). Exposure data at admission and during hospitalization were extracted from medical records by trained physicians. We used multiple regression methods (adjusting for age, sex, and disease's duration) to estimate the association between exposures and the case-control status. We evaluated 110 cases and 217 controls (mean age: 35.0 vs. 18.9 [p<0.001]; disease's duration pre-admission: 5.9 vs. 6.7 days [p=0.562]). History of previous hospitalization (27.9% vs. 11.2%) and hypertension (17.8% vs. 1.4%); respiratory distress (38.5% vs. 5.2%) and impaired consciousness (32.1% vs. 20.6%), were more frequent in cases than controls (p<0.05). In a model based on medical history, hypertension but not diabetes increased the odds of mortality (OR: 12.3; 95%CI: 1.41, 108.3). Further, a model that included respiratory distress (OR: 9.54; 95%CI: 2.56, 35.5), impaired consciousness (OR: 3.72, 95%CI: 1.18, 11.7), and heart rate (OR: 1.54, 95%CI: 1.37, 1.74 [per 5 bpm]) at admission had excellent predictive accuracy (AUC: 0.94, 95%CI: 0.90, 0.98). During hospitalization, controls but not cases showed increments of systolic (1.7 vs. 0.7 mmHg/day, p=0.045) and diastolic blood pressure (1.5 vs. -0.1 mmHg/day, p<0.001), as well as platelet count (11,605 vs. -333 per mL/day). Our results highlight the importance of medical history and easily measured clinical indicators in triaging dengue patients for mortality in endemic areas.

1702

AN EVALUATION OF MOLECULAR DIAGNOSTIC TOOLS FOR TRAVELERS' DIARRHEA: THE HOSPITAL FOR TROPICAL DISEASES EXPERIENCE

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Travellers Diarrhoea (TD) is the most common illness reported in returned travellers to the UK. A wide range of pathogens including bacteria, viruses and protozoa are responsible for TD. There are limited UK-based data on the aetiology of TD in our returned traveller population. The highly sensitive and specific detection and quantification of pathogen-specific nucleic acids in faeces, alongside cost-effective high throughput screening, makes PCR desirable as a diagnostic tool in ascertaining the aetiologic agents and informing the clinical diagnosis of TD. We present the first UK-based data investigating the aetiology of TD in travellers presenting to the Hospital for Tropical Diseases walk-in travel clinic. The objectives of the study were to evaluate the utility of an enteric pathogen PCR panel developed by Public Health England to investigate the aetiology of TD compared to standard microscopy and culture-based methods. A total of 124 unique stool specimens collected from immunocompetent patients presenting with symptomatic TD were processed using single and multiplex PCRs. Gastrointestinal pathogens were detected in 52% (64/124) of stool samples. The primary pathogens causing TD in this cohort were Giardia lamblia (12%) and norovirus (12%), followed by enteropathogenic *Escherichia coli* (EPEC) (4%), enteroaggregative *E. coli* (EAEC) (4%), Shigella spp. (2%), rotavirus (2%) and Entamoeba histolytica (1%). PCR diagnostics identified nine extra cases of giardiasis (increasing case detection from 5% to 12%) and a single case of amoebic dysentery. Use of PCR allowed identification of the viral causes of TD and showed greater sensitivity in the identification of parasites, which has public health and clinical implications. In conclusion, PCR diagnostics improved the detection of enteropathogens, allowing better assessment of the aetiology of TD. These important pilot data show that there is clearly a role for the routine use of molecular diagnostics in the clinical assessment of TD in the UK.

1703

RAPID, AUTOMATED EXTRACTION AND PURIFICATION OF NUCLEIC ACIDS FROM PATHOGENS DIRECTLY FROM WHOLE BLOOD SAMPLES

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Syndromic, PCR-based diagnostics that identify multiple pathogens in a single sample are quickly replacing the time-consuming gold-standard methods of culture, plating, and microscopy. While whole blood is an ideal sample type to use with this technology due to its richness in diagnostic targets, it is also rich in PCR inhibitors. Thus, whole blood samples typically require extensive sample processing and nucleic acid extraction before analysis. We developed the FilmArray® System (BioFire Diagnostics) to automate and integrate sample processing and nucleic acid extraction with nested multiplexed PCR to identify multiple pathogens in a single sample. Presently, there are four FDA-cleared FilmArray® panels that directly test nasopharyngeal secretions, blood culture media, stool, and cerebral spinal fluid. We are currently working to expand our first whole blood-based product, the Biothreat-E test for Ebola virus, to include other pathogens that cause febrile illness. We report the efficacy of FilmArray® sample preparation for a variety of intracellular and extracellular blood pathogens including Gram-positive and Gram-negative bacteria, parasites and viruses simultaneously from a single 200 µL whole blood sample. In addition, the Injection Vial allows dried blood spots to be used directly in the system without any added extraction steps. In whole blood, the estimated LOD was 1 x10^3 CFU/mL for *E. coli* and S. agalacatiae, and between 1 and 15 TCID50/mL for enterovirus, human parechovirus and herpes simplex virus 2. Extraction efficiency was between 25-90% depending on organism and was comparable to stand alone extraction systems. PCR inhibition was undetectable or at a low level that did not affect sensitivity. Additional evaluation with more diverse pathogens is ongoing and includes the causative agents of malaria, chikungunya, Zika, dengue fever, visceral leishmaniasis, leptospirosis, and Salmonella. This abstract contains data that have not been reviewed by regulatory agencies.

1704

PREVENTING *MYCOBACTERIUM LEPRAE* - ASSOCIATED DISABILITY: IDENTIFYING SOCIAL AND CLINICAL FACTORS ASSOCIATED WITH NERVE DAMAGE IN AN ENDEMIC AREA OF BRAZIL

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Hansen's disease (leprosy) remains a significant cause of morbidity and disability, with India and Brazil carrying the highest number of cases worldwide. *Mycobacterium leprae* infection affects skins and nerves and can cause permanent disabilities, which can have lasting effects on individuals' health and productivity. Addressing these preventable outcomes from various angles is crucial. We hypothesize that socioeconomic variables, such as income, occupation, and education level are associated with disability in patients with Hansen's disease (HD). Between July and December 2015, we enrolled patients at an HD reference clinic in Belo Horizonte, Minas Gerais, Brazil to identify variables associated with morbidity of HD. Patients with multibacillary disease were recruited, a questionnaire on several demographic & socioeconomic variables administered, and data abstracted from the medical chart. A

cross-sectional analysis was performed to determine associations with Grade 1 or 2 nerve disability according to World Health Organization (WHO) criteria. Seventy- three patients were enrolled (73% male). The majority of patients had nerve damage with Grade 1 disability found in 19 (26%) patients and Grade 2 in 29 (40%). On univariate analysis, older age (p=0.048) and lower education levels (OR = 5.4; 95% CI 1.4, 22.9) were associated with disability. Occurrence of reactions, clinical type of HD and other clinical and demographic variables were not found to be associated on preliminary analysis. Overall, our patients had a high burden of nerve damage consistent with prior studies in endemic areas. Additionally, older age and lower education were associated with disability grades of 1 or 2. While these findings are also consistent with other studies, overall data, to date, are limited and most of the literature has focused on clinical risk factors. These findings, along with planned multivariable analyses that may uncover other associations, will add to the body of knowledge on social factors associated with disability. This can then lead to strategies to target at-risk groups to reduce the burden of disease from this debilitating infection.

1705

NODDING SYNDROME/EPILEPSY IN THE SANAGA RIVER BASIN (CAMEROON): AN UNNOTICED EPIDEMIC?

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Nodding Syndrome (NS) is a severely debilitating form of epilepsy affecting children between the ages of 5 and 15 years in Northern Uganda, South Sudan and Tanzania. Unconfirmed cases of head nodding have also been reported in Cameroon and the Democratic Republic of Congo. The cause of the disease is unknown and there is no cure. Evidence suggests that there is an association between epilepsy/NS and onchocerciasis. However, relevant factors for the development of the condition are urgently needed. In order to explore these further, ethnographic research was carried out in 5 villages in the onchocerciasis-endemic Mbam Valley of the Sanaga river basin, Cameroon. Participant observations, in-depth interviews, informal conversations and focus group discussions suggested that there was a sharp increase of epilepsy about 40 years ago. Reports from older residents (50 years +) showed that epilepsy was uncommon during the 1970's, and that its prevalence increased dramatically during the 1980's and has decreased in recent years. These findings suggest the existence of environmental or social triggers for the occurrence of epilepsy and potentially NS. Relevant factors may be: (i) the construction of dams upstream of the study area (affecting the seasonal population dynamics of blackflies, and increase the transmission of onchocerciasis); (ii) changes in the patterns of human-water contact; (iii) changes in climate (rainfall and temperature); (iv) changes in nutritional habits and the variety of foods available; (v) the annual mass ivermectin treatment through the African Programme for Oncocerciasis Control (APOC) launched in the late 1990's. These factors may be key in determining the reported sudden occurrence of epilepsy/NS and need to be assessed further to contribute to the identification of the causes and conditions under which NS develops and becomes epidemic in certain locations and at specific times.

1706

HELMINTHS AND UNDERNUTRITION: FACILITATORS OF *MYCOBACTERIUM LEPRAE* MORBIDITY OR INNOCENT BYSTANDERS?

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While 30-50% of patients with Hansen's disease (HD) suffer from immunological Type 1 and Type 2 reactions that can lead to irreversible nerve damage, large gaps in knowledge exist about susceptibility to these complications. We hypothesize that helminthic co-infections and micronutrient deficiencies may be risk factors for reactions. Between July and December 2015, we performed a pilot case-control study at an HD clinic in Belo Horizonte, Minas Gerais, Brazil. Adult patients with multibacillary disease were recruited and were considered cases if they had an active Type 1 (T1R) or Type 2 reaction (T2R) or controls if free of reactions. Data were abstracted from the medical chart, and a demographic questionnaire was administered. Stool was collected for ova and parasite testing and venipuncture was performed for *Schistosoma mansoni* serology, complete blood count, C-reactive protein, Vitamin D level, and biomarkers for iron and vitamin A status. Statistical analyses were performed with adjusted odds ratios calculated for T1R and T2R as separate outcomes, controlling for age, sex, race socioeconomic status, rural residence, type of clinical HD, bacillary index, presence of anemia, other co-infections and smoking status. Seventy-three patients were recruited with 73% male and an average age of 51.2 years. Helminth infections were found in 4 patients with reactions and 1 patient without reaction, with total prevalence of 6.9%. Helminth co-infections were not found to be associated with T1R (aOR =3.5; 95% CI 0.17, 73.15) nor T2R (aOR = 0.07; 95% CI <0.001, 80.49). Micronutrient results are pending. While this pilot study did not show a statistically significant association with helminth infections and reactions, the total numbers of co-infections were small. Given the overall prevalence of low socioeconomic status, micronutrient deficiencies may play a role in the risk of reactions in our study. The nutrition results, future epidemiologic studies on co-infections in areas with higher helminth endemicity and immune studies hold promise in identifying strategies to reduce the significant morbidity of reactions in susceptible populations.

1707

CHITOSAN MICROPARTICLES TO DNA DETECTION IN URINE SAMPLES

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Chitosan is the second most abundant natural polymer in nature, derived by partial deacetylation of chitin. Chitin is part of the support structure of many living organisms, such as arthropods (crustaceans and insects), mollusks and fungi. Chitosan is being widely studied because of their advantages of biocompatibility, high charge density and non-toxicity. During the last years, has been reported the use of chitosan particles has the ability of association to peptides, proteins, oligonucleotides, due to the abundance of amino groups in its structure, thus allowing adsorption. Urine is a valuable non-invasive sample, studies report the presence of DNA fragments in urine, however the low concentration is not detectable by conventional methods, an alternative is the use of chitosan biopolymer to concentrate the small amount of nucleic acids and their future application in the diagnosis of infectious diseases. We infected

urine samples with DNA and applied the particles chitosan, previously we analized the pH interaction with DNA, we evaluated by PCR in real time. Chitosan particles has efficient to capture DNA .We hope to find the use of the particles could be used as a biomarker through DNA in urine samples.

1708

ASSESSING POSSIBLE EXPOSURE TO ZIKA VIRUS IN A HOSPITAL POPULATION THROUGH A TRAVEL SCREENING QUESTION

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Responding to possible exposures to infections with potential importation, Mount Auburn Hospital incorporated a travel screening question "Have you traveled outside the US within the past 30 days" into patient registration for each visit. We describe characteristics of patients registered from November 1, 2015 to January 31, 2016 who responded "Yes" to estimate the number of patients potentially exposed to Zika virus. De-identified data collected prospectively in MIDAS database is analyzed. Services are in 5 categories: 1) Inpatients, 2) ED/ Walk-In Center, 3) Travel clinic 4) Other outpatients, and 5) Laboratory/ radiology. Destination countries are categorized by Zika transmission: 1) Confirmed autochthonous transmission in past 9 months [ECDC 041516], 2) Documented prior presence (virus or serologic evidence), and 3) No documented Zika. All analyses are performed using SPSS 17.0. Of 1267 total visits, 1065 with complete destination and diagnosis data were included.The mean age was 47y, 62% were female, 83% reported English as primary language, and 75% were white. The most common contact point was diagnostics (radiology/lab; n=584). Seventeen percent registered to the ED/ Walk-in clinic. Thirty-eight patients were admitted (3.6%). Top destinations were Canada (n=132), Mexico (n=79) and France (n=78).310 patient visits reported travel to countries with Zika transmission (29%). The Caribbean was the most visited region with active Zika transmission (n=168). Among 660 female patient visits, 51% (n=341) were of child-bearing age (15-49y; WHO criteria). Ninety-seven of 341 (28%) patient visits reported travel to an active Zika transmission area. Fifty-three visits were by pregnant women. Eighteen pregnant women had visited Zika transmission area (1st trimester=12; 2nd trimester=4; trimester unknown=2).A travel screening question at patient registration allowed for analysis of potential Zika exposure. We found over a quarter of patients had visited Zika transmission areas. A significant number of women were of child-bearing age and most pregnant women who visited Zika areas were in their first trimester.

1709

PODOCONIOSIS: GENETIC PREDISPOSITION NORTHERN PROVINCE, RWANDA, AFRICA

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Non-filarial elephantiasis (also known as podoconiosis) is a noninfectious neglected tropical disease caused by prolonged exposure of bare feet to irritant volcanic soils. It is a disabling and debilitating condition that left untreated can lead to the severest stage of lymphedema (elephantiasis). The Imidido Project based in Musanze Town, Rwanda, Africa has been providing care for those suffering with Podoconiosis since 2009. Through data gathering during the clinic registration process, the research indicates that there is a genetic predisposition to susceptibility of acquiring this condition through chronic exposure to irritant volcanic soils. To date, we have registered 342 patients with Podoconiosis in various stages of advancement within the Northern Province of Rwanda Africa. Of these 342 patients, 183 (53.5%) indicated there were other family members that were suffering from this condition, including grandparents, parents, and siblings. The remaining 159 (46.5%) patients reported no familial link to the condition. The genetic linked group of high risk individuals has been a focus of our prevention education program in an effort to reduce the number of new cases in Rwanda, Africa.

1710

SYMPTOMS AND CLINICAL CORRELATES AS A PREDICTIVE MARKER FOR THE OUTCOMES IN TROPICAL FEVER: A TWO YEAR RETROSPECTIVE STUDY FROM CENTRAL INDIA

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There has been a resurgence tropical fever in India. Management protocols at our specialist centre have been aligned to the tropical fevers management guidelines issued by Indian Society of Critical Care Medicine. We aim to study the trends in symptomology and relate with predictive outcomes. We retrospectively evaluated the cohort of tropical fever patients including dengue, malaria, rickettsial infections, leptospirosis, typhoid, bacterial sepsis and viral infections. The syndromic approach with presenting feature as acute undifferentiated febrile illness was utilised to identify patients from the SCOUT integrated database of our tertiary care hospital. ANOVA was used for stratified analysis. Symptoms and outcomes were retrospectively corroborated as a predictive tool across 281 patients admitted during 2014-15. Year 2014 n= 180, 2015 n=101, Males n = 182, Females n=99. Overall, mortality rate was 4.62% (n=13; 2014 n=7, 2015 n=6). 38.7% (n=109) were within 20 km vicinity and included treatment naïve patients. Majority (n=172) were referred from remote, rural geography and had already received primary care for the severe grade symptoms. Stratified analysis was performed across the duration of hospital stay, age, symptoms, GCS on admission, diagnosis, complications ($p<0.0001$). Hospital stay was < 10 days in 89.6% of patients, 49.8% were < 5 days in hospital. Mortality rate was 6.42% with length of hospital stay of <5 days Vs 17% in 16-20 days. Patients with breathlessness at admission had highest mortality 20% (n= 6/30), followed by cough and cold 6.4% (n=2/31), headache 5.6%, vomiting 5.38%, abdominal pain 5.17%. 95% (n=267) had GCS > 8 at time of admission with mortality rate of 4.12%. ($p<0.0001$). Highest mortality (29%) was noted in dengue with IgM/IgG (2/7) followed by *Plasmodium vivax* 20% (1/5). Patterns and the outcomes of tropical fever could be the benchmark for the rest of the country. The early triage of tropical fever patients could optimise the utilisation of the hospital resources. Symptoms at hospital admission with the clinical correlates could be an important predictor for outcomes.

1711

COMPREHENSIVE CARE OF CHAGAS DISEASE IN A NON-ENDEMIC COUNTRY: THE EXAMPLE OF SPAIN

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Spain is the most affected country of Chagas disease (CD) in Europe, and the second non-endemic country globally (after US). Europe still faces an underdiagnosis of 90%. Among population from endemic areas, lack of knowledge, stigma and fear are still linked to CD. Community health activities are needed in order to reach population at-risk (par) and to overcome barriers for diagnosis and treatment. Activities have been performed synergistically by several institutions/organizations, in different Spanish regions. Highlighted: - CD patients' associations since 2008: Barcelona (ASAPECHA), Valencia (ASAPECHAVAE) and Murcia (ASAPECHAMUR). - Catalonian Expert Patient Program® on CD: initiative started in 2011 within the Chronic Disease Program. It aims to boost responsibility of patients for their own health and to promote self-care. Results: 15 participants completed the program. Knowledge about disease improved after sessions. - Mothers committed to Chagas' disease: taking action here and there®: community health workers (CHW) specialized in CD's training program, performed in Madrid in 2013. A second edition is currently in progress. Results (2014-2015): 1,401 par informed (185 in Bolivia); 60 par phoned the free-phoneline (900 103 209), asking mainly where to go for testing; 50 accompanied to the consultation by CHW; more than 7,000 received informative material. - Community screening campaigns performed in non-clinical settings on Sundays, on the occasion of CD International Day or Bolivia National Day's celebrations. Prior to the event, intense communicational campaigns are led by CHW and patients' associations. Results (2012-Feb. 2016): 3,474 par were screened in Barcelona, Madrid, Valencia, Murcia and Alicante; 775 were positive (CD prevalence 22.3%). - Access to treatment. 2013-2015: more than 4,000 treatments were administered among 155 healthcare centers all over the country. CD requires interdisciplinary approach including prevention, control, strategies and programs, being CHW and patient's associations key factors. Spain has reduced underdiagnosis and offers comprehensive care for CD patients.

1712

IMPACT OF MICRONUTRIENT SUPPLEMENTATION COMBINED WITH MALARIA CHEMOPREVENTION ON MALARIA, ANAEMIA AND COGNITIVE DEVELOPMENT IN EARLY CHILDHOOD: FINDINGS FROM A CLUSTER RANDOMIZED STUDY IN SOUTHERN MALI

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Early childhood is a time of rapid growth and development and public health interventions during this period could yield substantial benefits across several developmental areas: physical, cognitive and linguistic. Iron is important in brain function, and interventions that reduce iron-deficiency and anemia may improve cognitive function and learning. A randomized intervention study was undertaken to examine the combined impact of two newly-recommended interventions in early childhood: seasonal malaria chemoprevention and home fortification with micronutrient powders. Although each intervention has previously been shown to improve malaria morbidity, anemia and/or physical growth in children, the impact of combining these two complementary interventions is not known. No previous studies have examined effects on cognitive and linguistic development. A cluster-randomized controlled study of this combined strategy has been carried out in 60 rural communities in southern Mali since 2013. Children aged less than 5 years living in the 30 intervention communities receive seasonal malaria chemoprevention during the months of peak malaria risk, followed by daily supplementation of micronutrients for four months each year. Children living in control communities receive seasonal malaria chemoprevention only. The impact of the combined intervention after three consecutive years of implementation will be evaluated in May-June 2016 through cross-sectional surveys to compare malaria infection, nutritional indices and cognitive performance in children aged 3 and 5 years living in intervention and control communities. The results of this evaluation will be presented and discussed.

1713

A COHORT STUDY TO ESTIMATE THE RISK MERS-COV POSES TO TRAVELERS TO THE MIDDLE EAST

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Since its appearance in 2012, the Middle East respiratory syndrome coronavirus (MERS-CoV) has emerged as a serious public health threat of global concern. As of September 2015, the World Health Organization has been notified of 1,626 laboratory confirmed cases and the case fatality rate is estimated at approximately 36%. Beyond its high fatality rate, significant concern lies in the potential for MERS-CoV to spread beyond the Middle East, as was recently witnessed in the Republic of Korea which saw an outbreak of 185 confirmed cases and reported 36 deaths. The

spread of MERS-CoV may be facilitated by high population mobility and mass gatherings such as the Hajj pilgrimage that an estimated two million Muslims make each year to the region most impacted by the virus. Indeed, two of the most frequently visited cities during the Hajj (Mecca and Medina), have contributed nearly 10% of the known cases to the present epidemic. Despite the global concern over the virus and its potential for spread, many questions about MERS-CoV remain unanswered such as the number of asymptomatic cases that go undetected by current surveillance activities. Here, we describe an established multi-year cohort of pilgrims departing for Hajj from Malaysia, a country that sees an annual average of 20-25,000 Muslims make the pilgrimage each year. Within this cohort, pre- and post-pilgrimage serological analysis is paired with questionnaire data to estimate the risk of exposure to MERS-CoV during Hajj and assess its potential to spread beyond the Middle East.

1714

ROTAVIRUS AMONG MEDICALLY-ATTENDED CHILDREN YOUNGER THAN FIVE YEARS OF AGE WITH AND WITHOUT DIARRHEA IN LIMA, PERU FOLLOWING UNIVERSAL ROTAVIRUS VACCINE IMPLEMENTATION

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Before the monovalent vaccines Rotarix™ was added to the national immunization program in 2009, rotavirus A (RVA) was the leading cause of acute gastroenteritis among children in Peru. Although vaccination confers a high level of protection against several genotypes, continued monitoring of the prevalence of circulating strains that could affect vaccine efficacy is recommended. A case-control study was conducted in children younger than five years of age with and without diarrhea seeking medical care at Instituto Nacional de Salud del Niño in Lima, Peru between October 2013 and May 2015. Clinical data was gathered to determine the severity of gastroenteritis episodes, and stool samples were collected. Presence of stool RVA was determined using RT-qPCR, and positive samples were genotyped by a multiplex hemi nested PCR assay. During the study period, 1032 children (757 diarrhea cases 275 and controls) were analyzed. A total of 87.9% participants received the complete vaccine series. Prevalence of RVA was higher among cases (8.7%) than controls (3.6%) (p=0.0086). The emerging heterotypic G12P[8] was the most prevalent (54.8%) genotype. Among cases, no difference in the clinical severity, using either the Vesikari or Clark scales, was observed between RVA positive and RVA negative children. RVA infection remains an important cause of acute gastroenteritis among Peruvian pediatric populations. The identification of new circulating genotypes and their association with more clinically severe symptoms should continue to be evaluated.

1715

PREVALENCE AND ASSOCIATED RISK FACTORS OF DIABETES, CHRONIC KIDNEY DISEASE, HYPERTENSION AND OBESITY IN THE PERUVIAN AMAZON: THE AMARAKAERI RESERVE COHORT STUDY

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The Peruvian Amazon is in the midst of an epidemiological transition. Large urban population centers are growing rapidly, with non-communicable diseases (NCDs) beginning to outpace incidence of infectious diseases. However, in rural and peri-urban environments, population-level disease burdens are undefined with both infectious and NCDs being reported in increasing numbers. In this study, we determine the prevalence of NCDs in rural areas of the Peruvian Amazon to observe differences in prevalence between males and females and between native and non-native communities, and to identify risk factors associated with hypertension, obesity, diabetes, and chronic kidney disease (CKD). We conducted a cross-sectional study of 2,268 randomly selected adults (18-96 years old) in 1,122 households in communities surrounding the Amarakaeri Communal Reserve in the southern Amazon region of Madre de Dios. Disease prevalence was estimated using a finite population correction. WHO/ISH risk prediction charts were used to indicate the 10-year risk of a fatal or non-fatal major cardiovascular event. Comparing males vs. females, prevalence rates were: 7.0% vs. 1.8% for hypertension; 19.7% vs. 35.7% for obesity; 1.7% vs. 2.5% for diabetes; and 10.2% vs. 3.3% for CKD. Significant differences between males and females were detected for obesity, hypertension, and CKD prevalence. Among adults positive for diabetes or hypertension, 30.7% self-reported having diabetes, and 18.6% having hypertension. Multivariate analyses indicate that education, sex, and community location were important risk factors for each of these NCD outcomes. Physical activity and waist circumference were additional risk factors for hypertension and obesity. Only 3% of the population was at moderate or high risk for a major cardiovascular event. NCD burden is high and differential among males and females. Risk factors identified in this region indicate that disease burden may increase and have severe cardiovascular consequences. As many of these risk factors are modifiable, interventions should be implemented immediately to lower the NCD burden in this region.

1716

DEVELOPMENT AND PRELIMINARY CLINICAL EVALUATION OF A MOBILE TECHNOLOGY FOR DIARRHEAL DISEASE OUTBREAK MANAGEMENT

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The emergence of mobile technology offers new opportunities to improve access to clinical guidelines, especially in resource-limited settings during acute health crises. We conducted a multi-year design initiative to determine how best to adapt diarrheal disease outbreak management guidelines to smartphones. End-user design sessions with medical staff in rural Bangladesh resulted in the development of a rehydration calculator for use during initial resuscitation and a surveillance platform for real-time syndromic reporting and data visualization. The calculator was evaluated in a pre/post pilot study at a generalizable government district and sub-district hospital in Northern Bangladesh in an area prone

to cholera outbreaks (Netrokona). Inclusion criteria were patients with uncomplicated diarrheal disease (>= 3 loose stools per 24 hours) and an age >= to 2 months. The baseline and interventional arms were six weeks each. The primary outcome was adherence to guidelines for antibiotics (azithromycin for moderate and severe dehydration for suspected cholera), zinc (<5 years) and intravenous (IV) fluids. A total of 327 and 521 patients were enrolled during the baseline and interventional arms. For the district and sub-district sites, guideline adherence increased for antibiotics (13% to 82%, p<0.01; 63% to 99%, p<0.01, respectively), zinc (82 to 89%, p>0.01; 91 to 98%, p>0.01; respectively) and the use and administration of IV fluids. No adverse events related to the intervention were detected during admission and at 10-days post discharge. The surveillance platform (aka Outbreak Responder) was durable and reported clinical and laboratory endpoints in real-time. A randomized control trial is in development to accommodate for study limitations that included the lack of an independent control and implementation challenges. In this study, we report the successful technical first steps towards a smartphone-enabled platform for diarrheal disease outbreak management.

1717

DIFFERENTIAL CLINICAL AND LABORATORY CHARACTERISTICS AMONG ADULT DENGUE PATIENTS WITH DIABETES

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Dengue results in significant public health burden globally. It is usually a self-limiting disease, but about 1-5% of those symptomatic dengue infections results in dengue hemorrhagic fever, dengue shock syndrome (WHO 1997) or severe dengue (WHO 2009). Diabetes has been significantly associated with severe dengue progression. However, there is a lack of understanding of the differential clinical, laboratory, and immunological characteristics of these high risk group of dengue adult patients at presentation and during hospitalization, that potentially may provide insights in the disease pathogenesis of the virus and provide guidance on improved clinical management. Dengue patients with diabetes were significantly associated with hypertension, hyperlipidemia and severe dengue outcome. Several warning signs such as abdominal pain, clinical fluid accumulation and hematocrit rise and rapid platelet count drop were significantly associated with dengue patients with diabetes. Levels of white blood cells and neutrophils were significantly associated with dengue patients with diabetes. Immunologically, several chemokines and cytokines were significantly associated with dengue patients with diabetes. In conclusion, dengue patients with diabetes may have different immune responses against dengue virus, resulting differential clinical manifestations and disease severity. Much more immuno-pathogenesis studies are still required to provide understanding of how diabetes pre-dispose a patient with severe dengue outcome.

1718

ANTHELMINTHIC SCREENING FOR PARASITIC NEMATODES

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For many parasitic diseases, high-throughput phenotypic screening is an important tool in finding new drugs. Some of the most important parasitic diseases are caused by nematodes. However, these parasitic nematodes are not typically amenable to high throughput screening. Due to the ease of its maintenance and suitability for high throughput assay, the nematode *Caenorhabditis elegans* is instead used. To address whether *C. elegans* is a good model for nematode drug discovery, we compared the drug susceptibility of *C. elegans* relative to the human hookworm nematode parasite *Ancylostoma ceylanicum* at several developmental stages using a library of FDA approved drugs. I will present results of these studies that point to how well *C. elegans* efficacy correlates with hookworm efficacy and how early larval stages (easier to get) correlated with adult stages (more representative of what stage is targeted in human therapy). In addition, we are working on moderate-high throughput system for screening adult parasites. Using Union Biometrica, Copas, worm sorter we were able to sort adult parasites into 384 well format. Here I will discuss the capabilities of this system as well as how we are building de novo, in collaboration with the Albrecht laboratory at WPI, an imaging and image analysis platform for screening adult parasitic nematodes against large drug libraries.

1719

WHAT'S IN A NATIONAL PLAN OF ACTION? EVALUATING PROGRESS TOWARD GLOBAL CONTROL OF SOIL-TRANSMITTED HELMINTHIASES?

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Soil-transmitted helminthiases (STH) are estimated to affect more than 2 billion people worldwide. In effort to minimize this disease burden, the World Health Organization (WHO) has outlined milestones to monitor the progress of global STH control. Specifically, WHO has advocated for the development of national plans of action (PoA) for integrated control of neglected tropical diseases (NTDs) by all countries requiring preventive chemotherapy (PC) for STH by 2015. To measure progress on this indicator, we collected national PoA from WHO, national programs, and implementing partners. WHO indicates that 84 national PoA exist; of these, we were able to confirm and analyze 41, representing 40% of the 102 countries requiring PC for STH in 2014. All available PoA included STH control. Yet, a substantial proportion failed to address key roles for intersectoral collaboration with education, water and sanitation, and nutrition sectors. Although WHO recommends deworming to reduce morbidity from STH in both preschool-age children (PSAC) and school-age children (SAC), the majority of available PoA did not address PSAC as a target population. Our findings suggest that developing a national PoA is an effective step in STH control. Of countries with reported STH treatments for SAC in years prior to and during an active PoA (N=33), the average national coverage increased by 15.4% (95% CI: 6.8 - 24. 0%) under PoA implementation. However, even with this increase, only 16 of the 41 countries with available PoA reported coverage greater than 75% in the year 2013 or 2014. Our analysis is limited by the difficulty in collecting PoA. However, to date, this is the first collective review of available PoA for integrated control of NTDs. Most notably, our review suggests that if WHO milestones on STH control are to be met, improved efforts in developing and updating national PoA may be required.

1720

PREVALENCE OF MALARIA, GEOHELMINTHS AND ANAEMIA AMONG SCHOOL CHILDREN IN MUHEZA DISTRICT

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Malaria and intestinal helminths are an important public health issue with malaria-geohelminths co-infections commonly occurring in school aged children. A consequence of these co-infections in humans is anaemia. This cross sectional study aimed to determine the prevalence of malaria, geohelminths, co-infections and anaemia and associated factors among school children. The prevalence of malaria was 21.5% (82/381), (95% CI: 20.5 to 24.1) geohelminths (6.7%) 26/387 (95%CI: 8.4 to 12.9), co-infections (malaria-geohelminths) (1.8%) 7/381 (95%CI: 1.7 to 2.1) and anaemia was (39.1%) 149/381 (95%CI: 37.2 to 51.7). Non-use of insecticides treated nets (aOR 4, 95% CI: 2.24 to 8.51 P= 0.0012) was associated with malaria infection. Eating unwashed raw food (aOR 2.9,

95%CI: 1.9 to 9.2 P-value = 0.032) and not washing hands before eating (aOR 5.81, 95% CI: 1.92 to17.54 P-value =0.0002) were associated with geohelminth infections. Malaria and anaemia are prevalent in the study area while geohelminths and co-infections among school children are low. Further studies are required to explore the reasons why primary school children do not use insecticide treated nets and hygienic practice.

1721

CRYSTAL PROTEIN CRY5B AS A NOVEL AND POWERFUL ANTHELMINTIC

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Soil-transmitted helminths (STHs), most notably, hookworms, whipworms, and *Ascaris*, are nematodes that infect more than 1.5 billion of the poorest people and are amongst the leading causes of morbidity worldwide. Only two classes of de-worming drugs (anthelmintics) are available for treatment, and only one is commonly used in mass drug administrations. New anthelmintics are urgently needed to overcome emerging resistance and to produce higher cure rates. Crystal (Cry) proteins, in particular Cry5B, made by *Bacillus thuringiensis (Bt)* are promising new candidates. Cry5B has excellent anthelmintic properties against many free-living and parasitic nematodes, including *in vivo* efficacy against multiple STH infections in rodents (*Heligomasmidoes polygyrus* and *Ancylostoma ceylanicum*) and in pigs (*Ascaris suum*). An enormous challenge for STHs, very different from most diseases worked on in the developing world, is the requirement that therapies be very cheap (the people infected are very poor and current drugs costs pennies a dose), massively scalable (over 4 billion people are at risk from infection), and have a long shelf life in harsh environments, that have high temperature and humidity and no cold chain. We will update our progress in several key areas. We will present new data on the *in vivo* activity of Cry5B against a major human parasite of humans. We will also present data on the whether or not the immune system is required for Cry5B action *in vivo*. We will also present on our development efforts to produce a deployable version of Cry5B that is cheap, safe, scalable, and stable. These efforts are focused on bacterial engineering, expression, and formulation, and we believe we hit upon a novel bacterial expression system that meets these key requirements.

1722

PLANT DERIVED COMPOUNDS AS 'RESISTANCE-BUSTING" ANTHELMINTIC DRUG

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There is an urgent need for new therapies for parasitic helminthic diseases affecting 1.5-2 billion people worldwide due to the threat of wide-spread resistance development to existing treatments and due to problems of incomplete efficacies. Plants and plant secondary metabolites have been used historically to treat STH infections. Although they can be effective, we hypothesize that the active ingredients in plants may be absorbed prematurely, which limits their efficacy. Our hypothesis is that modern formulation techniques could be used to overcome limitations. We screened a number of plant extracts and metabolites for anthelmintic activity *in vitro* against adult stages of the hookworm and whipworm parasitic nematodes *Ancylostoma ceylanicum* and *Trichuris muris*. Here we will present results of this work, which shows the promising potential for some of these as pan-nematode anthelmintics. This work has allowed us to classify plant materials into at least two groups based on their *in vitro* killing kinetics. We have also shown that some are effective against an albendazole-resistant *Caenorhabditis elegans* strain suggesting that they may play an important role in overcoming helminthic drug resistance. We will also present our work on optimizing lead formulations *in vitro* and *in vivo* in animal models of parasitic nematode infection in order to overcome the challenges and realize the potential of "resistance-busting" plant-based anthelmintic therapies.

1723

INVESTIGATING THE DIFFERENTIAL IMPACT OF SCHOOL AND COMMUNITY-BASED INTEGRATED CONTROL PROGRAMS FOR SOIL-TRANSMITTED HELMINTHS IN TIMOR-LESTE: THE (S)WASH FOR WORMS PILOT STUDY

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Soil-transmitted helminth (STH) infections remain a significant global health issue, with an estimated 1.45 billion people infected worldwide. Water, sanitation and hygiene (WASH) interventions are thought to be important in sustainable STH control, alongside regular distribution of anthelminthic drugs. Currently, large-scale STH control programs are most often targeted to children, through school-based delivery systems. However, a recent meta-analysis shows greater reductions in STH prevalence in children following community-wide deworming, compared to child-targeted deworming. The (S)WASH for WORMS pilot study aims to compare the impact of school- and community-based integrated WASH and deworming programs on STH in school-aged children. This pilot study includes six remote communities in Timor-Leste. STH prevalence and intensity were measured in school-aged children at baseline in June 2015, using both a flotation-based microscopic technique and a quantitative PCR technique. All communities then received a WASH and deworming program at the primary school, and three communities additionally received a community-wide WASH and deworming program. STH prevalence and intensity will be re-evaluated in May 2016, six months after anthelminthic delivery. Cumulative incidence and intensity of STH infections at six months will be compared between the two study arms. At study baseline, 522/563 (91%) children present were recruited for the study. Stool samples were obtained for 483/522 (93%). STH prevalence was 37.7% (95%CI 33.2-42.3%) using microscopy, and 50.3% (95%CI 45.7-54.9%) using quantitative PCR. In summary, baseline results show that this pilot study achieved high participation rates and that STH are prevalent among school-aged children in Timor-Leste. Preliminary analyses suggest that quantitative PCR is more sensitive than microscopy for diagnosing STH infections. This presentation will discuss the pilot study in more detail, and will include final results comparing the two study arms.

1724

IS THERE EVIDENCE THAT THE SEASONAL TIMING OF MASS DE-WORMING FOR *ASCARIS* IS IMPORTANT?

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Regular mass treatment programs are commonly used in areas of high *Ascaris* prevalence. It is well known that that seasonal variables can affect maturation and mortality of *Ascaris* eggs, yet the potential implications for incidence, transmission and control are poorly understood. A recent field study of 477 individuals in Sri Lanka has shown significant correlation between *Ascaris* infection rates and a number of seasonal variables, including temperature. To demonstrate the implications that seasonal variation in climate could have for mass treatment, and the potential for intervention optimization, we have used statistical modeling techniques to simulate the results of seasonal timing of mass chemotherapy in different settings. Using historical experimental data on *A. suum* eggs to fit relationships between temperature and egg developmental parameters,

we find that the optimal temperature for Ascaris eggs lies in the range of 25°C-30°C. Higher temperatures facilitate egg development, but temperatures above 30°C show a steep drop in the proportion of eggs that survive. Using these relationships, a mathematical model is developed to represent seasonal mean worm burden in a population under various mass treatment conditions. We demonstrate the ability of this model to predict seasonal *Ascaris* prevalence using historical data from a study of approximately 600 individuals in Korea, across six neighbouring villages. Applying this model to prevalence data from the Sri Lanka field study allowed us to predict the outcome of mass treatment programs with different annual timing. Results suggest that tuning treatment timing could have significant consequences for program impact, with the optimal annual treatment date providing up to a 55% comparative decrease in prevalence after four treatment rounds. Whilst different seasonal patterns would give different results, this implies that we may have previously under-estimated the importance of seasonality in driving Ascaris infections. Further investigation into seasonal timing of treatment could result in long-term global implications for helminth control and elimination programs.

1725

SPONTANEOUS SEDIMENTATION IN TUBE TECHNIQUE IS AS SENSITIVE AS KATO-KATZ FOR THE DIAGNOSIS OF SOIL-TRANSMITTED HELMINTHS AND SUPERIOR FOR THE DETECTION OF *STRONGYLOIDES STERCORALIS*: A COMMUNITY-BASED STUDY IN THE AMAZON BASIN OF PERU

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Soil-transmitted helminthiasis (STH) constitute a major health problem especially in developing countries, where the lack of parasitologists and limited laboratory resources may be contributing factors to underestimate the burden of disease by these parasites. According to the WHO, Kato-Katz (K-K) method is the *gold standard* for the diagnosis of STH; however its sensitivity has been reported as low when compare to other methods. This study aimed to compare the sensitivity of K-K against Spontaneous sedimentation in tube technique (SSTT); a low-cost and rapid diagnostic technique for the diagnosis of STH. Fresh stool samples from residents of a rural community in the Amazon (Peru) were collected and analyzed by both techniques within 6 hours from emission. In addition, Agar plate culture was used as the *gold standard* for the diagnosis of *Strongyloides stercoralis*. One hundred seventy stools samples were collected in this study, mostly children. Overall, the prevalence of STH was 24.7%. In an individual analysis, the prevalence of these parasites by means of K-K or SSTT was as follows: *Ascaris lumbricoides* (12.4% vs. 13.5%), *Hymenolepis nana* (8.2% vs. 7.7%), *Trichuris trichiura* (1.2% vs. 1.2%) and hookworm (1.2% vs. 2.4%). Furthermore, the prevalence of *S. stercoralis* through K-K, SSTT and Culture was 0% vs. 4.1% vs. 10.6% ($p<0.001$). In conclusion, SSTT was as sensible as K-K for the diagnosis of STH and superior for the detection of *S. stercoralis* larvae in this specific population. Our results suggest that SSTT may be an alternative for the diagnosis of STH in tropical areas, and this finding warrants especial consideration, given the high prevalence of *S. stercoralis* in such regions. In addition, the SSTT may need further modifications in order to quantify the intensity of infection. Major advantages of this technique are the simplicity (no need for centrifuge) and low cost.

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POTENTIAL IMMUNOLOGICAL MARKERS FOR DIAGNOSIS OF HUMAN STRONGYLOIDIASIS USING HETEROLOGOUS ANTIGENS

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Strongyloides venezuelensis is a parasitic nematode of rodents that is frequently used to obtain heterologous antigens for immunological diagnosis of human strongyloidiasis. The aim of this study was to identify antigens from filariform larvae of *S. venezuelensis* for immunodiagnosis of human strongyloidiasis. Soluble and membrane fractions from filariform larvae of *S. venezuelensis* were obtained in phosphate saline (SS and SM) and Tris-HCl (TS and TM), and were analysed by western blotting (WB). Different antigenic components were recognized by IgG antibodies from the sera of strongyloidiasis patients. Highest recognition was observed for a 30-40 kDa band in all antigenic fractions. This band was then excised and subjected to mass spectrometry for protein identification. Immunoreactive proteins identified in the soluble fractions corresponded to metabolic enzymes, whereas cytoskeletal proteins and galectins were more abundant in the membrane fraction. Thus, these results represent the first step towards identification of *S. venezuelensis* antigens for use in immunodiagnostic assays for human strongyloidiasis.

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THE EFFECT OF MATERNAL POSTPARTUM DEWORMING ON INFECTION STATUS, ANEMIA AND FATIGUE

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Anemia and fatigue are common consequences of infection with intestinal parasites in women of reproductive age living in parasite-endemic areas. To date, no previous study has evaluated symptoms of fatigue in infected individuals using standardized scales. A randomized controlled trial conducted in 2013-2014 in Iquitos, Peru recruited 1010 mother-infant pairs. One objective of the study was to determine the effectiveness of maternal postpartum deworming on the prevalence and intensity of intestinal parasites, anemia, and fatigue in lactating women up to 6 months postpartum. Following delivery, women were randomly allocated to receive single-dose deworming (albendazole) or matching placebo. At 6 months postpartum, mothers provided stool specimens for detection of intestinal parasite infection, and finger-prick blood samples for assessment of blood hemoglobin concentration. The Fatigue Assessment Scale (FAS) was used to ascertain the self-reported presence of physical and cognitive symptoms of fatigue. A total of 970 (96.1%) participants attended their 6-month follow-up visit. The risk of parasite infection at 6 months postpartum was significantly lower in the group who received albendazole compared to placebo (RR: 0.5; 95% CI: 0.4, 0.6). At 6 months postpartum, no statistically significant benefit of deworming on maternal anemia (48.1% *vs.* 48.6%) or elevated fatigue (61.3% *vs*. 64.1%) was observed. Results were similar when analyses were restricted to mothers who tested positive for helminth infection at baseline. In the present study population, where baseline soil-transmitted helminth infection prevalence and intensity were low, deworming was highly effective at reducing the

burden of infection at 6 months postpartum; however, benefits in terms of maternal anemia or fatigue could not be detected at this time point. Further research is needed to determine which interventions, either during pregnancy or during the postpartum period, provide the most benefit to mother and infant at this critical time.

1728

SYSTEMATIC REVIEW AND META-ANALYSIS OF SOIL-TRANSMITTED HELMINTH TREATMENT EFFICACY STUDIES AND THE CASE FOR SHARING INDIVIDUAL PATIENT DATA

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In 2014, over 271 million schoolchildren were treated with benzimidazoles as part of the World Health Organization's plan to scale up mass drug administration (MDA) programmes targeting the soil-transmitted helminthiases (STHs) caused by roundworm, whipworm, and hookworms. There is consensus that drug efficacies should be monitored for signs of decline that could jeopardise the long-term effectiveness of MDA strategies. Efficacies are mostly calculated and reported as averages in groups of patients. However, heterogeneities in trial design and reporting hinder straightforward meta-analysis of these data, which could otherwise be used to explore varying efficacy among populations with different MDA histories or particular sub-populations of interest. Some heterogeneity issues could be avoided if individual participant data could be accessed, as this would facilitate the execution of standardized, state-of-the-art statistical analyses. Such data would also allow examination of the distributions of individual responses to anthelmintic drugs, offering a more sensitive means to identify reduced efficacies potentially caused by emerging drug resistance. To assess the trial landscape, we systematically search the STH literature for published anthelmintic trials. We collate locations, study sizes, methodologies, reported drug efficacies and other aspects of the reported data. We quantify these characteristics and create an overview of the variety therein, exploring the limits to analysing aggregated data. The results indicate the volume and characteristics of individual patient data that may exist and could be used to create a database on the efficacy of the anthelmintics that are the cornerstone of MDA targeting STH infections.

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USING TRANSMISSION MODELS IN STUDY DESIGN: DETECTING ELIMINATION AND THE IMPACT OF PRE-EXISTING TREATMENT PROGRAMS

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The DeWorm3 project aims to investigate the feasibility of eliminating soil transmitted helminth (STH) infection using mass drug administration, in particular by leveraging existing treatment programs such as for lymphatic filariasis (LF). The project uses mathematical models of transmission to aid study design. An important component of this work is to identify a statistic to identify when sufficient treatment has been delivered to achieve long-term elimination. We discuss a number of possible candidates, their sensitivity, specificity and their requirements in terms of sampling strategy. We also examine the potential impact of existing LF treatment platforms on the possibility of STH elimination and its detection. We investigate how STH elimination efforts can best be coordinated with such programs to maximise the possibility of success, particularly in cases in which LF programs have achieved their targets and are being discontinued.

1730

COMPARISON OF KATO-KATZ, MINI-FLOTAC AND MULTI-PARALLEL REAL-TIME POLYMERASE CHAIN REACTION TECHNIQUES FOR DETECTION OF SOIL-TRANSMITTED HELMINTHS IN FEIRA DE SANTANA, BRAZIL

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Soil-transmitted helminth (STH) infections, primarily caused by the roundworm *Ascaris lumbricoides*, the hookworm species *Necator americanus* and *Ancylostoma duodenale*, and the whipworm *Trichuris trichiura,* affect over 1 billion people, especially in warm, moist climates. Current STH control efforts in Brazil are conducted using passive surveillance and incidental case finding, such as by the Schistosomiasis Control Program, which is limited to schistosomiasis endemic areas, and this leaves STH infections under-notified. Diagnostic testing for the STH relies mainly on the WHO recommended Kato-Katz method, which has been shown to lack sensitivity. Other economical, feasible, and more accurate diagnostic methods are needed to detect and combat STH, especially in areas of low endemicity. In the city of Feira de Santana, Brazil, we collected human stool from four different areas of the city, one rural site, two peri-urban sites and one urban site. We compared the traditional Kato-Katz thick smear to two newer diagnostic methods, the mini-FLOTAC kit and a multi-parallel quantitative polymerase chain reaction (qPCR) technique. The mini-FLOTAC kit allows for quick analysis of fresh or preserved feces with minimal equipment needed. The multi-parallel qPCR can accurately detect and quantitate parasites within the stool with high specificity and sensitivity, and is optimized to allow for inexpensive analysis of each sample. All three diagnostic methods were analyzed for both parasite detection and quantification. Both the mini-FLOTAC and multi-parallel qPCR offer feasible, higher-accuracy diagnostics, which will enable a shift away from morbidity control and towards elimination, especially in areas of low STH endemicity.

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ANTIPARASITIC METABOLITES OF *DALEA* SPP (PLANTAE, FABACEAE)

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About a billion people are infected worldwide with hookworms. These intestinal parasites are the major cause of iron-deficiency anemia, weight loss, stunted growth and malnutrition in endemic areas. Despite control strategies using mass drug treatment combined with water, sanitation and hygiene, hookworm infection remains a major public health threat to the overall wellbeing of populations in endemic countries. Moreover, there is increasing concern with reports of lower efficacy of current drugs used in the treatment of hookworms and other soil-transmitted nematodes. Consequently, there is an urgent need of new tools to control the transmission of these helminths. We have explored the anthelminthic potentials of natural products from plants including those of the genus *Dalea* spp. The activity of *Dalea* metabolites against the adult *Ancylostoma ceylanicum* hookworm was assessed using an *ex vivo* assay. Whole extracts, chromatographically-enriched fractions and pure compounds from eight plant species (Dalea spp) were evaluated. Worm mortality due

to plant extracts varied from 0 to 100% by day 5 post-incubation. Some extracts recorded 0% worm survival i.e 100% mortality by 24 hours after incubating worms and plant products. Toxicity of pure compounds to mammalian cells was evaluated by flow cytometry and their effects on cell proliferation by BrdU. In vivo evaluation of pure compounds using our hamster laboratory model of hookworm infection is also underway. Detailed results will be presented.

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A COMPARATIVE ANALYSIS OF STOOL PRESERVATION TECHNIQUES FOR THE MOLECULAR DETECTION OF SOIL TRANSMITTED HELMINTHS

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Accompanying the growing successes of the world's soil transmitted helminth (STH) treatment and elimination programs is an increasing need for sensitive and species-specific molecular diagnostic techniques. While the continued development of new tools is helping to meet this need, sample preservation remains a largely understudied, yet crucial aspect of the stool-based diagnostic process. Diagnostic test sensitivity is not only critical at the level of the individual, but is equally important for programmatic decision making. However, poor sample preservation renders all testing insensitive, making this aspect of diagnostics one of utmost importance. Accordingly, we have performed a comparative analysis of eight different literature-cited stool preservation techniques. Utilizing human stool samples spiked with hookworm eggs at concentrations of 60 eggs per gram (epg) and 200 epg, samples subjected to each preservation methodology were analyzed for the presence of detectable levels of hookworm DNA following storage for one, two, four, and eight weeks at 32 °C. Results have indicated variable preservation efficacy across methodologies as measured by the real-time PCR-based detection of parasite DNA. These results will help program managers to more appropriately structure their future survey efforts, allowing for the more informed balancing of performance needs and budgetary constraints.

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COST ASSESSMENT OF FIVE PARASITOLOGICAL TECHNIQUES FOR THE DIAGNOSIS OF *STRONGYLOIDES STERCORALIS*: EVALUATION IN A HIGHLY ENDEMIC REGION

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Inexpensive, easy to carry out and highly sensitive diagnostic techniques are needed to estimate the real global burden of *Strongyloides stercoralis* infection. This is especially important in tropical areas, where high prevalence rates of this parasite have been reported. We conducted a cost assessment for the detection of *S. stercoralis* by five parasitological techniques: direct microscopic examination (DME), Kato- Katz (K-K), Spontaneous Sedimentation in Tube (SSTT), Modified Baermann technique (MBT) and Agar plate culture (APC), by using fresh stool samples collected from an Amazonian rural community in Peru. The cost of a single sample was estimated considering the costs of laboratory materials and the time consumed in each technique. Out of 234 samples, 207 met the criteria for analysis (sufficient stool amount for examination). The prevalence of *S. stercoralis* was 0.48% (n=1) by DME, 0% (n= 0) by K-K, 3.86% (n= 8) by SSTT, 9.66% (n=20) by MBT and 10.14% (n=21) by APC. The total cost per a single exam was 0.48$ for DMS, 0.59$ for K-K, 0.70$ for SSTT, 0.79$ for MBT and 1.18$ for APC. The cost per case of *S. stercoralis* detected was 99.36$ (DME), 18.11$ (SSTT), 8.18$ (MBT) and 11.63$ (APC), respectively. Analysis of cost per positive case was not performed on K-K, as no larva of *S. stercoralis* was detected by this method. In conclusion, MBT and APC represent low-cost techniques when taking into account the rate of cases detected. However, in poor-resource settings where technicians and laboratory resources are scant, MBT and SSTT may represent cost effective parasitological techniques for the detection of *S. stercoralis*.

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PREDICTING INFECTION DISTRIBUTION AND BURDEN OF DISEASE USING SPATIOTEMPORAL MODELS FOLLOWING A SEVEN YEAR MASS DRUG ADMINISTRATION PROGRAM AND LONGITUDINAL STUDY IN BURUNDI: 2008 - 2014

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Spatiotemporal models (STM) of soil transmitted helminth infections (STH-I) prevalence provide important information for developing effective control strategies. We aimed to quantify the impact of a 7-year mass-drug administration programme (MDA) on the geographical distribution of STH-I and burden of disease (BOD). Longitudinal data on STH-I were collected from 2008-2014 in Burundi. Remotely sensed environmental variables (EV) were used. Associations between EV and STH-I were assessed using univariate and multivariate generalised linear regression models. Bayesian binomial geostatistical models were built to quantify the propensity, cluster size and produce predictive STM for each STH species. Our study found that after accounting for EV, trends in spatial-dependency in STH-I still existed. Model validation found that STM for all parasites were accurate, with 95CI-ROC values between 0.3-1. Predicted infection rates/1000 children fluctuated in accordance with the prevalence. A. lumbricoides showed a maximum of 319/1000 in 2008, 239 in 2011 and 324 in 2014; T. trichiura presented 173 in 2008, 103 in 2011 and 225 in 2014; Hookworm presented 571 in 2008, 262 in 2011 and 219 in 2014. Our findings indicate that using BOD predictions, STM based on longitudinal data may be helpful in maximising the effectiveness of MDAs through optimized resource management.

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PSYCHOSOCIAL ADJUSTMENT IN PERINATALLY HUMAN IMMUNODEFICIENCY VIRUS INFECTED OR EXPOSED CHILDREN

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This study was undertaken to determine whether perinatal HIV infection/exposure adversely affected psychosocial adjustment (PA) between 6 and 18 years of life (i.e. during school-age and adolescence). We enrolled 58 perinatally HIV infected, 56 HIV-exposed uninfected and 54 unexposed controls from Kampala, Uganda. Perinatal HIV status was determined by 18 months old using DNA-polymerase chain-reaction test and was confirmed via HIV rapid diagnostic test at psychosocial testing when children were 6–18 years old. Five indicators of PA (depressive symptoms, distress, hopelessness, positive future orientation and esteem) were measured using validated, culturally adapted and translated instruments. Multivariable linear regression analyses estimated HIV-status related percent differences (β) in PA indicators and corresponding 95% confidence intervals (CI). During school-age and adolescence, positive outlook (β=-3.8, 95% CI:-7.2,-0.1) and self-esteem (β=-4.3, 95% CI:-6.7,-1.8) scores were significantly lower whereas depressive (β=11.4, 95% CI:3.3,19.5) and distress (β=12.3, 95% CI:5.9,18.7) symptoms were elevated for perinatally HIV-infected compared to unexposed controls and HIV–exposed uninfected children. Similarly, positive outlook (β=-4.3, 95% CI: -7.3,-1.2) and self-esteem was lower for exposed controls vs. HIV-unexposed children. Hopelessness was similar by perinatal HIV status. Likewise, the distress and depressive symptom levels were comparable for HIV-exposed uninfected and HIV-unexposed children. In conclusion, perinatal HIV infection predicted higher distress and depressive symptoms, while HIV-affected status (infection/exposure) predicted low self-esteem and diminished positive outlook in the long-term. However, HIV-affected status had no impact on hopelessness suggesting that psychosocial interventions as an integral component of HIV-care for infected children or primary care exposed uninfected children may improve PA and quality of life in these vulnerable groups.

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PERCEPTION OF HUMAN IMMUNODEFICIENCY VIRUS SCREENING AMONG PASSERSBY ALONG A STREET CONNECTING THE EAST AND WEST GATES OF THE ADVENTIST UNIVERSITY AT CARREFOUR, HAITI, AUGUST 2ND 5TH, 2015

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Screening for HIV can be a health indicator for the population. Boulevard Perin is the lane artery borrowed by students university. This alley is the junction between East and West exit out of the university campus. However, this artery is also used by local residents who consider this passage as a shortcut, Diquini and Bizoton. This survey was conducted among thoseestimate the importance ofscreening, and describe some associate risk factors. A descriptive survey conducted among 258 passers who borrowed this path on August 2nd5thin 2 hours of time. These passers-by were divided into three categories, those who enter into the eastgate, west gate, and those who were scattered elsewhere on campus. Questions were asked about their last date of testing andthought of importance of HIV screening. The data were collected on Google forms, processed and analysed on EPI info 7. 258 passerby, 129 (50%) were women. The average age of passerbyyears for a standard deviation of + / - 9 years. For marital status,(68.5%) were single, and 108(41.86%) ofto havethan one sexual partner. For the perception of HIV testing,80.71% (205) think it is important to get tested, though 63(29.72%) say they have never been screened.(10.85%) do not to recall the date of their last screening. In conclusion, this study allowed passerby to understand the importance of the testing. However, prevention campaigns should be conducted by Carrefour health officials in order to increase their screening frequency for HIV.

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PREVALENCE OF CERVICAL CANCER (CC) SCREENING AND THE ROLE OF KNOWLEDGE OF CC RISK AND SCREENING GUIDELINES FOR WOMEN LIVING WITH HIV IN LIMA, PERU

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Cervical cancer (CC) incidence of women in Peru is twice the worldwide average. Awareness by women living with HIV (WLHIV) of their increased risk and Papanicoloau (Pap) smear frequency is understudied, particularly in Peru. We assessed the prevalence of adherence to the recommended CC screening guidelines among WLHIV and if knowledge of CC risk and screening was a factor. 80 HIV-infected women receiving care at Via Libre, a HIV clinic, were surveyed from 2014 - 2015 by a self-administered questionnaire. Knowledge of CC for WLHIV was assessed through 4 questions regarding CC risk and Pap smear frequency for WLHIV. A correct response was worth 1 point leading to a possible score out of 4 per participant. "Adequate knowledge" was judged as ≥ 3/4 correct answers. The Wilcoxon rank sum test was used for bivariate analysis. Nearly all (91.3%; 73/80) WLHIV were enrolled in a HAART program. 21.3% (17/80) of WLHIV were adherent to the guidelines by obtaining a Pap smear within 12 months of study enrollment. 78.8% (63/80) were not adherent by having their most recent Pap smear outside of 12 months, including 11.3% (9/80) who had never been screened. The median composite score for knowledge of CC risk and screening was 3 (IQR 2 -4) for adherent WLHIV and 2 (IQR 1-3) for non-adherent WLHIV. 58.8% (10/17) of adherent WLHIV met "adequate knowledge" criteria as did 41.3% (26/63) of non-adherent WLHIV. When asked how often WLHIV should get a Pap smear, 76.5% (13/17) of adherent WLHIV answered "Every year" correctly while 55.6% (35/63) for non-adherent WLHIV were able to. Overall, bivariate associations found between knowledge score and adherence were not significant. Prevalence of WLHIV who had on-time Pap smears was three times less than WLHIV who did not. Knowledge of CC risk and screening guidelines influenced adherence as more adherent WLHIV met the criteria for adequate knowledge. Larger studies of this population are needed to assess the educational, social, and structural barriers to screening and potential benefits of HIV and gynecological care integration services.

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HIV/NCD INTEGRATED CARE: A LITERATURE REVIEW

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With advances in antiretroviral therapy, people living with HIV (PLHIV) are living longer lives and HIV itself is becoming a chronic disease. Since PLHIV are living longer lives, they are increasingly contracting other diseases such as diabetes and hypertension. Despite the prevalent view that NCDs are ailments faced only by the wealthy, NCDs burden low-and middle-income countries (LMICs) at ever increasing rates. In fact, 80% of global deaths from NCDs occur in LMICs due to weak health systems, limited access to information, medication, and services. As individuals, communities, and clinics move towards treating HIV as a chronic illness, opportunities arise to deliver both NCD and HIV services in an integrated way and to build

stronger health systems capable of delivering more patient-centered care. Several health-focused organizations have pursued the opportunity to enhance patient care by integrating HIV and NCD services. The goal of this paper is to present findings from a literature review of these programs, identify best practices, and provide recommendations for policymakers. We studied 23 programs from 11 different organizations operating across Sub-Saharan Africa and examined 4 aspects of each program: which NCDs were addressed, what services were offered, the level of health system involved, and whether the programs engaged in policy work. Diabetes and cardiovascular disease were the most commonly targeted diseases, followed by cancer and mental health illness. Services commonly included screening, diagnosis, and treatment for NCDs in conjunction with existing HIV care. Most integration occurred at the lowest level of care including at the home, community and HIV clinic. Services were occasionally offered at the tertiary level as well. Few of the programs we studied engaged in policy interventions. Some programs, however, did engage Ministries of Health at the national level on NCD protocols. Linking HIV/AIDS and NCD care regimens is a new concept and more research is needed. This review is intended to aid policymakers and program implementers design further integrated programs and stronger health systems.

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HIV CO-INFECTION WITH *PLASMODIUM VIVAX* MALARIA AND OTHER TROPICAL INFECTIOUS DISEASES IN THE PERUVIAN AMAZON

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Interactions between HIV and other infectious diseases have the potential to alter course of disease, response to therapy, and epidemiology of some or all agents involved. In the tropics, geographic overlap of the "big three"–HIV/AIDS, tuberculosis, and malaria - result in millions of deaths and disability-adjusted life years annually. Loreto, a region in the Northern Peruvian Amazon, is responsible for 90% of the country's malaria burden and has the second-highest prevalence of HIV. In an attempt to understand the overlap of infectious diseases, we began a cohort study of outpatient HIV patients from Loreto Regional Hospital assessing infectious disease history, including malaria. Out of 337 enrolled patients, 33% were female and 75% defined themselves as heterosexual. Most (59%) attended high school and were employed (65%). The median years since HIV diagnosis was 3.83 years (IQT=1–7.83 years) and 278 (86%) had disclosed their status. Patients reported on average 2 (IQT=0-2) respiratory infections and 2.9 bouts of diarrhea (IQR=0-2) in the last year. Further, 162 (49.39%) reported having had tuberculosis, 120 (36.59%) hepatitis, and 51 (15.55%) dengue. Five individuals had cerebral toxoplasmosis and live with lasting neurological sequelae. Microscopy detected one case each of *Plasmodium vivax, P. falciparum*, and filaria. ELISA for *P. vivax* using PvMSP1-19 confirmed microscopy result and detected 14 low (OD 0.25-0.50) and 11 high positives (OD >0.50), a total of 25 positives by ELISA (7.65%). PCR confirmed both cases of malaria identified by microscopy and identified one unconfirmed case of *P. malariae*. Other ELISA *P. vivax* positives were PCR negative. Of the 27 malaria ELISA or PCR positives, 22 self-reported cases of malaria, 7 of which occurred less than 6 months of enrollment. Patient reports suggest multiple infectious diseases affect this population, especially individuals that live and work in peri-urban and rural environments. *P. vivax* malaria co-infection is frequent but its consequences are little known. Research should try to more accurately identify the interactions and burden of co-infections in this neglected population.

1740

PRESENTATION, ETIOLOGY, AND OUTCOME OF FEBRILE INDIAN PATIENTS DIFFERS BY HIV STATUS

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Despite a <1% HIV prevalence, India has the third largest burden of HIV worldwide. Acute febrile illness is one of the most common reasons for hospital admission in India, but the clinical differences between HIV infected and uninfected patients are unknown. Patients >= 12 years of age admitted to general medicine wards at BJ Medical College - Sassoon General Hospital, in Pune, India with >1 day of fever were enrolled into a prospective cohort between July 2013 and December 2015. We compared clinical characteristics, laboratory data, treatment course, and mortality between HIV positive and negative patients using fisher exact test and a multivariable logistic regression model adjusted for modified Apache II score, age, and sex. Of 970 participants enrolled, 127 (13%) had HIV; 37 were newly diagnosed. Median CD4 count was 161 cells/cumm; 61 (49%) were on ART. Vector-borne infections among patients living with HIV included dengue (n=5), malaria (n=2), chikungunya (n=2), and leptospirosis (n=1). Additional infectious disease diagnoses included bacteremia (n=6), pneumonia (n=7), meningitis (n=16), and microbiologically confirmed tuberculosis (n=10). Two patients with mosquito-borne illnesses also had microbiologically confirmed tuberculosis. Compared to patients without HIV, patients with HIV were more likely to have meningitis (13% vs 4%, $p < 0.01$), diarrhea (42% vs 16%, $p < 0.01$), tuberculosis (8% vs 1%, $p < 0.01$), and alcoholism (21% vs 10%, $p < 0.01$), and were less likely to have dengue (4% vs 24%, $p < 0.01$) and malaria (2% vs 8%, $p < 0.01$). Patients with HIV more frequently received fluoroquinolones (24% vs 10%, $p < 0.01$) and antituberculosis drugs (24% vs 4%, $p < 0.01$). Mortality was more than two times higher in HIV infected patients (adjusted odds ratio 2.1, confidence interval 1.1-3.8). People in India living with HIV who develop acute febrile illness more commonly have diarrhea, tuberculosis, and meningitis, and less commonly have mosquito-borne illnesses. Clinicians must recognize that patients living with HIV present differently with acute febrile illness, and are more likely to die.

1741

CONDOMLESS INSERTIVE ANAL SEX AND GENDER IDENTITY AMONG MEN WHO HAVE SEX WITH MEN IN TOGO

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Consistent condom use continues to be an effective preventive tool to control HIV transmission among men who have sex with men (MSM). Few studies have explored the association of condomless insertive anal sex (CIAS) and gender identity among African MSM. Our aim was to identify factors associated with CIAS among MSM living in Lomé and Kara, Togo. A total of 683 MSM ≥18 years of age were recruited using respondent driven sampling (RDS) for a cross-sectional survey (354/683 in Lomé and 329/683 in Kara). Participants completed a structured questionnaire and were tested for HIV and syphilis. Statistical analysis included t-test, RDS-weighted (RDS-w) proportions, bootstrapped confidence intervals and logistic regression models. Overall median age was 23.9 years; 62.8% were between 18 and 24 years (RDS-w Lomé=79.1%, RDS-w

Kara=55.5%, p<0.01). Most participants identified themselves as being males (RDS-w male=67.9% vs. 91.2%, p<0.01, female=18.1% vs.1.3%, p<0.01 and intersex=13.9% vs. 7.4%, p<0.01 in Lomé and Kara, respectively), were single/never married (RDS-w Lomé= 91.7% vs. Kara=95.4%, p=0.11), and reported their sexual identity as gay/homosexual (RDS-w Lomé=61.2% vs. Kara: 62.6%, p=0.59). Consistent condom use in the past 12 months was reported by 30/270 MSM who had insertive anal sex (RDS-w Lomé=30.5% and Kara=6.2%, p<0.01), 17/198 who had receptive anal sex (RDS-w Lomé=13.4% and Kara=not available (NA)), and by 100/223 who had vaginal/anal sex with a woman (RDS-w Lomé=58.6% and Kara=not available (NA)). HIV prevalence was 62/683 (RDS-w Lomé=10.4%, RDS-w Kara=0.2%, p<0.01). Multiple logistic regression analysis showed a positive association between CIAS and intersex gender (adjusted OR=3.0, 95% CI=1.2-7.4). In Togo, local cultural and social norms could increase the number of condomless insertive anal sex acts among MSM who self identify as being of intersex gender. Strategies to address gender inequity should be included in HIV prevention programs aiming to address the needs of MSM.

1742

COMPARISON OF ASYMPTOMATIC AND CLINICAL MALARIA FREQUENCIES BETWEEN HIV POSITIVE AND HIV NEGATIVE INDIVIDUALS LIVING IN GABON

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This study was undertaken to compare the frequency of clinical and asymptomatic *Plasmodium falciparum* parasitaemia between HIV-positive and HIV negative individuals living in Gabon. Prospective screening for malaria parasitaemia of HIV-infected Gabonese adults on antiretroviral therapy (ART) and HIV negative individuals were performed at two health centers and during a survey on malaria knowledge performed in asymtomatic volunteers in 2015. Clinical malaria was defined as fever with a positive blood smear, and asymptomatic malaria as a positive blood smear in the absence of fever and history of fever the preceeding 7 days. Data from three hundred thirty seven asymptomatic and 76 febrile HIV-1-positive patients were compared to those from 439 asymptomatic and 130 symptomatic HIV negative adults. Clinical malaria frequency was 31.6% among HIV positive and 29.3% among HIV negative patients. Asymptomatic malaria prevalence rate was comparable between both groups (9.6% in HIV positive and 8.4% in uninfected population). Cotrimoxazole prophylaxis was non significantly associated with a lower malaria prevalence : 4.3% versus 1.2% among asymptomatic HIV patients and 42.9% versus 27.8% in symptomatic ones. HIV1 infection is not associated with a higher frequency of asymptomatic or symptomatic malaria prevalence in patient on ART. The present results also suggest a protective effect of cotrimoxazole prophylaxis on malaria occurence in HIV-positive individuals.

1743

LEVERING HIV DIAGNOSTIC AND CARE INFRASTRUCTURE IN RWANDA TO ACCELERATE THE ROLL-OUT OF NEW PEDIATRIC TB TREATMENT FORMULATIONS

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Since the end of the genocide in Rwanda, the country has made impressive gains in health system strengthening. In particular, the rapid scale up of antiretroviral therapy has been a great success story for Rwanda. Tuberculosis remains an important health concern in sub-Saharan Africa, and infants and children in particular, are at the highest risk for severe forms of the disease because of immature immune systems. New pediatric formulations of anti-tuberculosis drugs have renewed calls for aggressive roll-out of an aggressive diagnostic and treatment campaign in affected regions to reduce the burden of TB among infants and children. The success of Rwanda's scale-up of ART can greatly inform the process of accelerated roll-out of new pediatric anti-TB formulations. Through a review of the history of HIV treatment scale-up in Rwanda, as well as a field assessment of current interventions and available evaluation data, we developed a framework to identify the successes of ART expansion in Rwanda and how lessons learned can be applied to the accelerated roll-out of new pediatric anti-TB formulations. Key themes of this framework identify a decentralized form of care administration and a centralized system of data collection that supports real-time monitoring and evaluation. Innovative health system financing strategies, including a universal community health insurance scheme, have improved access to primary care services for prevention and treatment of HIV and TB. Furthermore, cost-effective and responsive supply chains for drug delivery have permitted scale-up with limited stock outs. This framework of successes in the delivery of ART in Rwanda can be leveraged to improve pediatric care for TB immediately in Rwanda, and lessons learned can be applied to other countries with a high burden of infant and child TB across the sub-Saharan African region.

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SEXUALLY TRANSMISSIBLE INFECTIONS (STI'S) AMONG HIV CLIENTS ATTENDING AN URBAN UGANDAN HIV CLINIC

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Naguru Hospital was established as a Regional Referral Hospital in February 2012.In July2012, an HIV clinic was started to take care of the many patients who live with the infection in Kampala City and its surrounding areas. To date the cumulative number of clients stands at 6480 while the still active clients stand at 3750.We were concerned about the possible complications caused by sexually transmissible co-infections so we set out to find out their prevalence among our clients. Of the 6480 records looked at,139 had a clinical or laboratory diagnosis of an STI. We found the commonest diagnoses to be: Herpes Simplex 2 (47=33.8%) candidiasis (38=27.3%),syphilis(20=19.4%), other genital ulcer diseases-other than Herpes 2 (20=19.4%), genital warts(7=5%), gonorrhea (5=3.5%) , Hepatitis B(5=3,5%), Bacterial Vaginosis (1=.7%). Genital chlamydial disease was not identified by any of our clinicians implying that it was probably missed. A prospective study to ascertain the true burden of STI's among our HIV patients is required to avert complications and possible mortality from these treatable co-ailments.

1745

TRYPANOSOMA CRUZI INHIBITION OF SIRT1/PGC1 ACTIVITY CONTRIBUTES TO ANTIOXIDANT/OXIDANT IMBALANCE BUT NOT TO MITOCHONDRIAL BIOGENIC DEFECTS: BENEFITS OF SIRT1-TARGETED THERAPY IN CHAGAS DISEASE

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Increased oxidative/inflammatory stress and decreased mitochondrial function are the hallmarks of chronic chagasic cardiomyopathy. SIRT1/PGC1 signaling of NRF1 and Nrf2 regulates mitochondrial biogenesis and antioxidant response. C57BL/6 mice were infected with *Trypanosoma cruzi* (Tc) and monitored during chronic phase (~150 days post-infection). SIRT1 and PGC1 protein levels were normal; however, SIRT1 activity and PGC1α deacetylation (active-form) were decreased significantly in chagasic myocardium. Tc-infected mice were treated with SIRT1 agonist SRT1720 for 3-weeks after control of acute parasitemia (i.e. 45 days post-infection). SRT1720 therapy provided the maximal benefits in restoring the SIRT1/PGC1 activity, and subsequently left ventricular (LV) function (stroke volume, cardiac output, ejection fraction etc.) was significantly improved

in chronically-infected/SRT1720-treated mice. SIRT1-targeted therapy did not improve the PGC1/NRF1-dependent mitochondrial biogenesis (i.e., mitochondrial DNA content, expression of subunits of the respiratory complexes and mtDNA replication machinery) and the disproportionate synthesis of collagens and LV mass in chagasic mice. Instead, SRT1720 therapy restored the Nrf2 level and antioxidant capacity; and subsequently resulted in 2-10-fold inhibition of Tc-induced oxidative (H2O2 and advanced oxidation protein products), nitrosative (inducible nitric oxide synthase, 4-hydroxynonenal, 3-nitrotyrosine), and inflammatory (IFNγ, IL1β and TNFα) stress and inflammatory infiltrate in chagasic myocardium. These results suggested that Tc-inhibition of SIRT1/PGC1 activity inhibition was not the key mechanism in mitochondrial biogenic defects during Chagas disease. SIRT1/PGC1 activation enhanced the antioxidant capacity, and subsequently controlled the oxidative/nitrosative and inflammatory pathology and LV dysfunction in chronic chagasic cardiomyopathy. These findings indicate that activators of the sirtuin family of proteins will provide promising new therapeutic strategies for treating cardiac dysfunction in chronic chagasic disease.

1746

THE *LEISHMANIA* METAPHYLOME: A COMPREHENSIVE SURVEY OF *LEISHMANIA* PROTEIN PHYLOGENETIC RELATIONSHIPS

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Leishmaniasis is a neglected parasitic disease with diverse clinical manifestations and a complex epidemiology. It is known that the infecting *Leishmania* species is responsible for the distinct clinical presentations and treatment needs due to virulence factors that mediate host parasite interaction, infectivity and pathogenicity. However, our understanding of these species-specific adaptations and their evolutionary background is still limited. To improve our knowledge regarding the biology and adaptive mechanisms of different *Leishmania* species, we conducted a proteome-wide phylogenomic analysis to gain insights into *Leishmania* evolution. The analysis of the reconstructed phylomes (totaling 45,918 phylogenies) allowed us to detect genes shared in pathogenic *Leishmania* species, such as calpain-like cysteine peptidases and 3'a2rel-related proteins, or genes that could be associated with visceral or cutaneous development. Our findings demonstrated that gene duplication constitutes an important evolutionary force in *Leishmania*, acting on protein families that mediate host-parasite interactions, such as amastins, GP63 metallopeptidases, cathepsin L-like proteases, and our methods permitted a deeper analysis of their phylogenetic relationships. Our results highlight the importance of proteome wide phylogenetic analyses to detect adaptation and evolutionary processes in different organisms and underscore the need to characterize the role of expanded and species-specific proteins in the context of *Leishmania* evolution by providing a framework for the phylogenetic relationships of *Leishmania* proteins.

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POLYMORPHISMS IN CASPASE-1 ARE ASSOCIATED WITH CHAGAS CARDIOMYOPATHY IN SANTA CRUZ, BOLIVIA

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Trypanosoma cruzi infection is usually acquired in childhood in endemic areas. Chagas cardiomyopathy (CC) develops in 20-30% of infected individuals over decades. CC pathogenesis involves the host inflammatory response to *T. cruzi*, in which upstream caspase-1 activation prompts the cascade of inflammatory chemokines/cytokines, cardiac remodeling and myocardial dysfunction. To date, no reliable early biomarkers of CC risk have been identified. However, polymorphisms of caspase -1 have shown to be associated with susceptibility to myocardial infarction and cardiovascular death risk. We recruited infected (Tc+) patients (n=151) and uninfected (Tc-) patients (n=85) in a hospital in Santa Cruz, Bolivia, to examine the association of two caspase-1 single nucleotide polymorphisms (SNPs) with cardiomyopathy. Cardiac status was categorized as A: normal EKG without systolic dysfunction and/or segmental wall motion abnormalities; B: ECG with abnormalities consistent with CC but normal ejection fraction (EF); CD: systolic dysfunction (EF<50%). We compared A vs BCD (all cardiomyopathy) and A vs B (early cardiomyopathy) for Tc+ and Tc-. Genotypes were determined using Taqman probes via RT-PCR in peripheral blood DNA. Genotype frequencies were analyzed by 3 inheritance patterns (dominant, recessive, additive) using logistic regression adjusted for sex and age in the SNPassoc R package. Caspase-1 SNP rs501192 showed consistent differences in AA genotype frequencies in Tc+ BCD vs A (aOR 2.25 [0.83-6.08]) for the recessive model. The association was similar but stronger for Tc+ B vs A (aOR 2.84 [0.99-8.13]). No significant associations were found in comparisons within Tc- groups or between Tc- and Tc+ patients. Our data suggests that polymorphisms within caspase-1 play a role in CC development, identifying individuals with a higher risk of developing Chagas cardiomyopathy. Further investigation into genomic biomarkers may help identify Tc+ patients for whom intensified monitoring and early medical intervention could be beneficial.

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ARTEMISININ DIMERS AS PROMISING NEW DRUG LEADS FOR VISCERAL LEISHMANIASIS

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Visceral leishmaniasis is a major global health problem with more than 12 million confirmed cases, about 2 million new cases being added every year and more than 350 million people at the risk of being exposed to the disease. The disease is fatal if left untreated. The current choices of therapies are highly limited and suffer from severe drug-toxicities or have become ineffective due to drug-resistance. Recent studies at our laboratories have identified a few novel artemisinin dimers with

outstanding antileishmanial activities against intracellular amastigotes of *Leishmania donovani,* the clinically relevant parasite stages for the visceral leishmaniasis. Antileishmanial activities of these dimers are several folds better as compared to the current battery of clinically used antileishmanial drugs. These dimers do not show any toxicity on differentiated THP1 cells. Selectivity index (SI) has been calculated by comparing toxicity with antileishmanial activity. Dimer piperidine (IC50 0.073 µM, SI >198), dimer morpholine (IC50 0.007 µM, SI >2052), dimer valine (IC 50 0.060 µM, >230), dimer AB acid (IC50 0.013 µM, SI >999), dimer tryptamine (IC50 0.045 µM, SI >165), dimer oxime HS (IC50 0.062 µM, SI >219), dimer benzylamine (IC50 0.099 µM, SI >141) and dimer GABA (IC50 0.013 µM, SI >1086)) have been selected as promising leads for extended evaluation. The *in vitro* antileishmanial activity of the lead analogs has been further confirmed by THP1 cells-*L. donovani* amastigotes digital image analysis counting of intracellular amastigotes. Artemisinin the parent drug from this class do not show noticeable antileishmanial activity up to 35 µM concentration. This indicates selective leishmanicidal action of the artemisinin dimers. The artemisinin dimers thus offer promising leads, which can be further optimized and developed as oral treatments for visceral leishmaniasis.

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IDENTIFICATION AND CHARACTERIZATION OF "YINP", A NOVEL GENE INVOLVED IN *LEISHMANIA* PATHOGENESIS. A POTENTIAL NEW TARGET FOR DRUG DISCOVERY

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Leishmaniasis is a neglected tropical disease caused by *Leishmania* spp. Current drug therapies are unsatisfactory due to their toxicity, long treatment courses and development of resistance. To improve existing treatments, the identification and characterization of novel therapeutic targets based on parasite genes involved in *Leishmania* pathogenesis is essential. One of these genes is "YinP", identified in our research group. It may play a role in the acquisition of infectivity by *Leishmania* promastigotes. This gene is highly conserved and has demonstrated to be involved in several cellular processes such as embrionary progress, ribosomal biogenesis, cellular proliferation, and genetic transcription. Our assays showed that YinP reaches its highest expression level in metacyclic promastigotes, the infective stage. Furthermore, we have performed several experiments to analyse the infectivity of parasites overexpressing YinP. Our data reveal a dramatic increase of the ratio of infection as well as a higher replication rate whithin macrophages exposed to *Leishmania* overexpressing YinP. All together, these results strongly suggest a relation between YinP gene expression and leishmaniasis pathogenesis. Moreover, to localize YinP expression in the leishmanial cell, two plasmids were constructed: pXG-mCherry12-YinP and pXG-mCherry34-YinP. In these vectors, YinP gene was inserted directly next to the gene for fluorescent protein (mCherry), to generate fluorescent fusion proteins expressed in the parasites. Fluorescent microscopy disclosed that red fluorescence of mCherry fused with YinP was localized only in a part of nucleus. Therefore, our results showed that YinP protein is expressed in the nucleus. Further experiments need to be performed to analyze if such expression is nucleolar. Finally, in order to validate YinP as a therapeutic target, we are carrying a screening of new compounds that have shown promising leishmanicidal activity. Our preliminary results have shown that YinP overexpressing parasites seem more resistant to these drugs. Therefore, this gene may be a new and good molecular target.

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PROTEOMIC ANALYSIS OF PLASMA-DERIVED EXTRACELLULAR VESICLES IN NATURAL INFECTIONS OF *PLASMODIUM VIVAX*, *TRYPANOSOMA CRUZI* AND *FASCIOLA HEPATICA*

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Chagas disease, fasciolosis and the malaria caused by *Plasmodium vivax*, are three neglected tropical diseases responsible for millions of infections worldwide, including disease and death. Our present research effort is focused on the molecular characterization of plasma-derived extracellular vesicles (EVs) from these neglected diseases to further study their role in intercellular communication, and the use of their selective-cargo as novel therapeutic agents and diagnostic markers, as reported previously. EVs were obtained from infected patients (malaria and Chagas) and infected cows (*Fasciola hepatica*), as well as from healthy volunteers and animals as controls of specificity. Size exclusion chromatography (SEC) using sepharose CL-2B, commercially available as qEV columns (iZONTM), was used for isolation of EVs. Molecular characterization was done by a bead-based assay with antibodies against tetraspanins CD9 and CD81, by cryo-transmission electron microscopy (cryo-TEM), and by Nanoparticle Tracking Analysis (NTA). An extensive LC-MS/MS analysis was performed using different mass spectrometers and algorithms. The proteomic composition of plasma-derived EVs from samples of these neglected tropical diseases contain parasite-specific proteins. Their identities and possible roles facilitating studies on mechanistic insights of pathology, as well as antigen discovery for vaccination and disease biomarker identification will be presented and discussed.

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IDENTIFICATION OF NOVEL INHIBITORS OF *LEISHMANIA* INITIATION FACTOR 4A AND ASSESSMENT OF THEIR BIOLOGICAL EFFECTS ON PARASITE GROWTH

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Leishmaniases constitute a group of parasitic vector borne diseases. They correspond to major public health problems that are neglected and ill controlled. The identification of novel targets and drugs for chemotherapy constitute a research priority. Different pieces of evidence point to *Leishmania* initiation factor eIF4A (LeIF), member of the DEAD-box protein family, as a potential drug target. Through a structure-based drug discovery process, we previously identified a novel inhibitor (C208) of LeIF ATPase activity (Harigua-Souiai et al., submitted). In the present work, C208 was used as a bait to search chemically related molecules. The Morgan fingerprints were used as chemical descriptors to assess the similarity between the molecules. Their use as criteria to identify moieties potentially responsible for bioactivity was validated by our group and in drug discovery in general, as reported previously. This virtual chemical

screen led to the identification of 28 analogous molecules. In a first step, fifteen of them were screened out of which a second hit was retained (CS48). It demonstrated modest levels of inhibition of LeIF activity compared to C208. Both C208 and CS48 were tested for their effects on the *Leishmania* promastigote viability. Their respective IC_{50} values were of 3 and 4 µM. Currently, as the ATPase screening is tedious, requesting high amounts of recombinant protein and compounds, we are testing the remaining 13 compounds for their effects on promastigote viability, then we will evaluate the active molecules for their effect on the ATPase enzymatic activity of LeIF. Five molecules were tested so far and a third hit (CT7) having an IC_{50}=1 µM was retained. At the IC_{50} concentration, these compounds had no to un-significant cytotoxic effect on human cells. Selectivity index (SI) was established for the C208 (SI= 6.6) in a preliminary way. Compound C208 and its analogs may constitute a novel route for drug design for anti-*Leishmania* treatments. Further work will be undertaken to understand the mode of binding and interaction between LeIF and its most promising inhibitor.

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GENOTYPING OF PANAMANIAN *TRYPANOSOMA CRUZI* STOCKS USING A MAXICIRCLE MULTILOCUS SEQUENCE TYPING APPROACH

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Chagas disease, caused by *Trypanosoma cruzi* is a major health problem in Central America leading to significant economic losses in this region due to reduced productivity from early-age mortality and disability. Differently from the rest of Central America, the main Chagas disease vector in Panama is Rhodnius pallescens, a sylvatic triatomine closely associated with "royal palm" trees. It has been suggested that the geographical heterogeneity in Chagas disease pathology and clinical outcome is related to parasite genotype. The present work provides information on *T. cruzi* discrete typing unit genotypes circulating in endemic areas of Chagas disease in Panama. Thirty *T. cruzi* stocks isolated from persons with different clinical profiles, as well as from vectors and different mammalian hosts were included in the study. Initial molecular analysis using mini-exon, heat shock protein 60 and glucose-6-phosphate isomerase nuclear markers confirm that DTU TcI was the predominant genotype found. To further evaluate intra-DTU diversity within Tc1, we use a multilocus sequence typing (mtMLST) approach. Six maxicircle gene fragments were amplified: ND1 (NADH dehydrogenase subunit 1), COII (cytochrome c oxidase subunit II), MURF1 (Maxicircle unidentified reading frame 1, CYT b (cytochrome b), 12S rRNA and 9S rRNA, coding regions. For each isolate, maxicircle sequences were concatenated according to their structural arrangement. Phylogenetic analysis was performed by two types of applied phylogenetic techniques: neighbor-joining method and Bayesian inference. The results showed a low level of diversity within *T. cruzi* Tc1 isolates. The epidemiological significance of these findings is discussed.

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EVALUATION OF RHOB GENE SILENCING MEDIATED BY SHRNA ON INFECTION PHENOTYPE OF U937 CELL DERIVED MACROPHAGES INFECTED WITH *LEISHMANIA (VIANNIA) BRAZILIENSIS*

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The limited knowledge of the *Leishmania* - macrophage interaction has interfered with the development of control strategies for leishmaniasis. *Leishmania* is able to modulate the immune response of the host cell by altering macrophage gene expression during infection. In a previous work, RhoB a gene that encodes for a small GTPase, was differentially expressed between non-infected and infected macrophages with L. (V.) braziliensis. The regulation of RhoB expression is related to sterols processing, pathway altered during *Leishmania* infection. The aim of this study was validate expression levels of RhoB in infected macrophages with L. (V.) braziliensis and determine the effect of RhoB silencing mediated by shRNAs on infection rate and burden load of infected macrophages. The expression levels of RhoB were measure by qRT-PCR from 0 to 120 h after *in vitro* infection. Lentiviral transduction was used to generate U937 derived cell lines bearing the constructs encoding for the expression of shRNAs against RhoB. RhoB gene silencing and active form of RhoB were assessed using western blot and pull down assay respectively. Generated cell lines with highest levels of RhoB silencing were infected and colored with fluorescent stain to determine the effect of RhoB silencing on infection rate and burden load. Results show that RhoB expression did not change between 0 and 24 h after infection while it is up regulated between 48 and 96 h. In the other hand, RhoB silencing is associated with decreased infection rate and burden load suggesting that RhoB could be related with the establishment of infection.

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TRANSCRIPTIONAL PROFILE OF HUMAN WHOLE BLOOD CELLS STIMULATED WITH SOLUBLE *LEISHMANIA* ANTIGENS

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Visceral leishmaniasis (VL), caused by *Leishmania infantum*, affects many people around the world. It has been estimated that only 10% of infected individuals will develop symptomatic disease and this might be primarily due to host-associated factors (*i.e.* environmental and genetic factors). This study aimed to assess the global gene expression pattern of peripheral blood cells, when challenged with Soluble *Leishmania* Antigen (SLA) stimulus. Whole blood cells from symptomatic VL patients (n=7) and individuals within 4-12 months of VL recovery (n=4) were divided into two groups, non-stimulated and SLA-stimulated, upon four hours of incubation with and without SLA, respectively. Total RNA were extracted and hybridized against ~ 48,000 probes using a microarray platform (HumanHT-12 BeadChip). The recovered group had 604 differentially expressed (DE) genes whereas symptomatic group had 128 DE genes. Overall, symptomatic individuals were able to express nearly 20% (118/604) of those genes expressed by the recovered individuals, suggesting an incomplete response under SLA stimulus. These 118 common genes were most from Cell chemotaxis (GO:0060326) and Chemokine receptor binding (GO:0042379) pathways, as shown by enrichment analysis. The list of DE genes for the recovered group was enriched with genes from Response to lipopolysaccharide (GO:0032496), TNF and Toll-like receptor signaling pathways (KEGG:04668 and 04620). Of note, 10 genes were exclusively changed in symptomatic group, which included apoptosis genes (*TNFRSF10B, CARD9*) and a galactoside-binding protein (*LGALS3)*. With a two-fold change increase under SLA stimulus (adjusted p=0.01), *LGALS3* might play important role in VL pathogenesis by regulating the response to innate immunity signals as well as proinflammatory cytokine production.

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BACTERIAL CO-INFECTION IN MURINE CUTANEOUS LEISHMANIASIS

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Leishmaniasis is a collection of human protozoan diseases caused by *Leishmania* inoculated through sand fly bites. Cutaneous leishmaniasis

(CL) causes localized skin lesions often followed by ulceration. In some human infections, response to treatment is best when initiated after ulceration. However, immune responses associated with ulceration and their effects on the course of disease are relatively unexplored. CL ulceration introduces bacteria into subdermal layers, and secondary bacterial infections are common. Thus, we hypothesized that bacterial effects are critical determinants of CL outcome. *Staphylococcus aureus*, a bacterium commonly isolated from disrupted human skin, is often found in CL lesions. We hypothesized that bacteria present during *Leishmania* inoculation, both before and after ulceration occurs, activate inflammatory responses that augment inflammation and contribute to host control of parasitic infection. We injected *L. major, S. aureus*, or both intradermally into the ears of female C57BL/6 mice. We monitored inflammation and parasitic burden by lesion volume, histology, and qPCR through 4 weeks post-infection (p.i.). During the first week of infection, there were greater lesion sizes in co-infected ears compared to ears injected with *S. aureus* alone, whereas ears injected with *L. major* alone do not yet form lesions. At 4 weeks p.i., we observed greater lesions sizes and decreased parasite burden in ears co-infected with a higher dose of *S. aureus* compared to ears infected with *L. major* alone or co-infected with a lower dose of *S. aureus*. Overall, these data suggest that co-infection with *S. aureus* increases inflammation, contributing to control of *L. major* burden but potentially greater lesion pathology. Identifying the specific inflammatory responses activated by skin microbiota in leishmaniasis, such as inflammasomes, may lead to novel therapeutic approaches to parasitic infection.

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PHYLOGENETIC ANALYSIS OF RNA OF CRIMEAN-CONGO HEMORRHAGIC FEVER AND WEST NILE FEVER SELECTED IN KAZAKHSTAN

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Studies performed on 14 samples of CCHF RNA extracted from ticks collected in South-Kazakhstan Oblast and 3 samples of WNF RNA extracted from mosquitos caught in Western-Kazakhstan Oblast. Prior to study,RNA samples were modified into cDNA. Sequencing was performed with the use of BigDye® Terminator v3.1 Cycle Sequencing Kit according to manufacturer's instruction with following separation of fragments on automatic genetic analyzer 3730xlDNAAnalyzer. Nucleotide sequences obtained by means of direct and reverse primers were analyzed and combined in general sequence using SeqScape 2.6.0 software. Sequences of reference strains of corresponding virus deposited in international database NCBI were used as the matrix. Construction of dendrogramm was performed with Mega 5.0 software, equalization of nucleotide sequences was performed with Muscle algorithm, construction of phylogenetic trees was performed by means of Neighbor-Joining method. Performed sequencing of S-segment nucleotide sequence (on 7 samples of ticks), M-segment (1 sample) and L-segment (9 samples) CCHF genome and E-segment of WNF virus (on 3 mosquito samples). Comparison of nucleotide sequences indicated that the samples being studied are most contiguous to strains from Afghanistan, Pakistan, Oman, Tadzhikistan, Uzbekistan and China, and are related to *"Asia 1-2"*CCHF groups. Comparison of nucleotide sequences of E-segment of WNF (with the length around 800 nucleotide pairs) indicated that WNF RNA in studied mosquito samples from Western-Kazakhstan Oblast are genetically close to the RNA of Russian isolates.

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ANTI-*LEISHMANIA DONOVANI* ANTIBODIES ENHANCE PROMASTIGOTES INTERNALIZATION INTO HOST MACROPHAGE

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This study aimed to demonstrate the role of humoral immunity in *Leishmania* parasite internalization into host macrophages. First, informed consent sera were obtained from 67 parasitologically confirmed visceral leishmaniasis patients reporting to our field treatment centre, Eastern Sudan. Then following titre determination, sera that had a titre of >102,400 were selected for parasite coating. An *in vitro* parasite internalization system was developed to enhance the *Leishmania*/ macrophage interactions. The mean parasite number per monocytes was 626 ± 91 for antibody-coated *Leishmania donovani*, compared to 412 ± 70 uncoated isolates (p= 0.01). On the other hand, the percentage of infected cells was significantly higher for all antibody-coated isolates (100%) compared to uncoated ones (40%). This evidence of high infectivity probably points to the fact that anti-*Leishmania* antibodies facilitated the parasite uptake by host macrophages and monocytes-derived macrophages (MDM). Conclusion *Leishmania* spp. promastigotes preferentially infect host macrophages, where parasite internalization is facilitated by several host and parasite surface molecules. Moreover, the rate of parasite uptake by MDM was significantly higher compared to monocytes. This could be explained by the fact that the functional capabilities of fully differentiated macrophages differ from monocytes. In conclusion, host humoral immunity probably plays a pivotal role in *Leishmania* parasites internalization into host macrophages.

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THE ROLE OF IL-10 AND IFN-γ IN VIRULENCE OF DERMOTROPIC *LEISHMANIA DONOVANI* IN SRI LANKA

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Cutaneous leishmaniasis (CL) in Sri Lanka is caused by an apparently dermotropic variant of *Leishmania donovani*. The visceralization potential of *L. donovani* in Sri Lanka and determinants of disease outcome is not yet fully understood. The current study was aimed at determining visceralization potential, cytokine response during local *L. donovani* infection and to explore the effects of route of infection on disease outcome. Parasites isolated from an ulcerated lesion of CL patient were used to inject BALB/c mice via intra-dermal (ID) and intra-venous(IV) infections (6 each with 2 controls similarly treated with normal saline). Similarly another set of mice were infected intra-dermaly with *L. donovani* wild type strain (Ld1S). Mice were euthanized after 10weeks following inoculation. Both spleen and liver were removed and cultured. The effect of infection on the animal including its weight, spleen, parasitic load and induction of IFN-γ and IL-10 in splenocytes and lymph nodes of BALB/c mice were measured. Skin lesions were observed in the ear piece of ID-infected BALB/c mice (n=4/6) and none of them showed any sign of visceral infection. However, infection of spleen was evident in 4 out of 6 IV-infected mice. All BALB/c mice infected with Ld1S showed spleen infection. High parasite burden and IL-10 levels were observed in spleen and lymph nodes of the BALB/c mice infected with Ld-1S, IFN-γ was low in these cells. Moderate levels of IFN-γ was observed only in mandibular node and splenocytes of ID-infected (with local parasites) BALB/c mice, while minute levels of IL-10 was observed of these animals. Splenocytes and popliteal lymph node of IV-infected (with local parasites) BALB/c mice

showed a moderate level of IL-10 and low level of IFN-γ. Local strain of *L. donovani* has the capacity to establish infection in BALB /c mice, inducing visceral disease. The level of IL-10, IFN-γ and the route of infection play a role in determining disease outcome. This study also may imply that, the local strain, though predominantly dermotropic in humans may acquire the ability to visceralize over time. The use of this model is being pursued for detailed investigation of this parasite.

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CUTANEOUS LEISHMANIASIS DUE TO *LEISHMANIA DONOVANI*: ROLE OF IL-4 AND IFN-γ IN LESION HEALING

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Sri Lanka is a newly identified focus of cutaneous leishmaniasis (CL) caused by the usually visceralizing *Leishmania donovani*. In situ cytokine expression plays a key role in the pathogenesis and lesion healing. This study describes the association between expression of Interleukin (IL)-4 and Interferon-gamma (IFNg) and time taken for lesions to heal. Skin biopsies from 58 patients with parasitologically or histopathologically confirmed CL and 25 healthy controls were collected in RNAlater and quantified for local tissue expression of IL-12A, IL-4, IL-10, IFNγ and TNF-α by real-time RT- PCR using SYBR green. Relative copy numbers were calculated by $2^{-\Delta\Delta Ct}$ method using β-actin as the reference gene and healthy controls as calibrators. Patients were treated with intra-lesional sodium stibogluconate. Correlation between cytokines and time taken to heal estimated with Spearman's rank correlation test. Study group consisted of 37 males (63.8%) and 21 females (36.2%) with a mean age of 35 years (SD=12.05, range 18-66) and a mean lesion duration of 6.75 months (SD=9.1, range: 1-48). Type of lesion varied from papules and nodules to non-healing ulcers. A total of 44 (75.8%) patients, consisting of 28 (63.6%) males and 16 (36.4%) females were followed up for time taken to heal. The mean treatment duration was 3.0 months (SD=1.75, range 1.5-8) and correlation coefficient between relative gene expression and time taken to heal for IL-12A, IL-4, IL-10, IFNg and TNF-α were 0.073, 0.321, -0.002, 0.257 and 0.155, respectively. A significant positive correlation was found between IL-4 and time taken to heal (p= 0.034). A tendency to have increased expression of IFNg was also observed though statistically not significant. Increased expression of both IL-4 and IFNγ are predictors of poor lesion healing in CL due to *L. donovani*.

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OXYGEN METABOLISM REGULATES MACROPHAGE SUSCEPTIBILITY TO *TRYPANOSOMA CRUZI*

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Macrophages (MΦ) are one of the early responders to control the causative agent of Chagas cardiomyopathy, *Trypansoma cruzi*. Infection and incomplete clearance of *T. cruzi* by MΦ result in parasite dissemination to peripheral tissues, and is a significant cause of chronic disease progression. Aerobic metabolism is often dependent on mitochondrial oxidative phosphorylation, and is associated with the activity of immunomodulatory macrophages (M2). Conversely, mitochondria-independent, glycolytic metabolism provides substrates to produce anti-microbial mediators by pro-inflammatory macrophages (M1). Reactive oxygen species (ROS) and nitric oxide (NO) are key M1 molecules for host defense against intracellular pathogens, which are associated with MΦ utilizing oxygen-independent metabolism. The susceptibility of macrophages to *T. cruzi* infection and incomplete clearance has been previously suggested to be due to lack of substantial pro-inflammatory activation of MΦ, however, the required mechanisms for a potent macrophage response for *T. cruzi* clearance remain unknown. In this study, we report a potent induction of the TNF-α pro-inflammatory cytokine, and deficient production of ROS and NO by macrophages infected with *T. cruzi*. Mitochondrial gene expression and cell respiration analysis suggested that *T. cruzi* infection elicit a metabolic response in MΦ which is similar to M2. These findings suggest that modulation of oxygen metabolism may improve the macrophage function for pathogen clearance to limit disease progression.

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MACROPHAGE CELLULAR IMMUNE RESPONSES IN CUTANEOUS LEISHMANIASIS AGAINST *LEISHMANIA DONOVANI*

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Leishmaniasis has a spectrum of manifestations including cutaneous, mucosal and visceral disease, caused by parasitic protozoa of the genus *Leishmania*. The clinical outcome of *Leishmania* is determined primarily by the infecting species and the immune response of the host. In Sri Lanka localised cutaneous leishmaniasis (LCL) is caused by *Leishmania donovani*; a visceralizing species. We hypothesized that the distinct alterations in the early immune response determines the outcome of infection observed in Sri Lanka. Aim of this study was to characterize the immune response in LCL by macrophages; which is central to both replication and elimination of the parasite. Peripheral blood mono nuclear cell (PBMC) derived macrophages from LCL patients (n=8) and healthy non endemic controls (n=8) were stimulated *in vitro* with *L. donovani* antigen (50µg/ml). The production of IL-10, TNFα, TGF β and Nitric Oxide (NO) were measured by ELISA and Griess test at predetermined time intervals. The two groups were compared using Student's T-test for parametric and Mann-Whitney test for non- parametric data. IL-10 production by patient macrophages was significantly higher (105.68±26.05vs 19.81±28.24pg/mL;p<0.01) at 72 hours but did not vary markedly at 24 & 48 hours. Production of TNFα by patients macrophages was significantly higher at 24 (15.63±16.44vs5.43±1.41;p<0.01),48(438.42±140.63vs30.06±24 .82;p<0.01)&72 hours (412.31±222.11vs14.41±11.68;p<0.01). TGF β production was higher at 24(1539.80±490.40vs1080.19±366.87)&48ho urs(1962.29±754.94vs1456.77±811.99)than the controls, but the values didn't vary significantly. Production of NO showed increased levels by LCL macrophages at 72 hours (5.40±1.15vs2.36±1.21; p<0.01). These data suggest IL-10; TNFα & NO play a role in determining disease outcome in LCL due to *L. donovani*. In contrast to TNFα, the contribution of IL-10, TGF - β and NO appear to be later in the infection. The findings should be interpreted in the context of changes in other imflammatory mediators, to better understand the underlying pathogenic mechanisms where a visceralizing *Leishmania* species is localized to the skin.

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IDENTIFICATION OF MICRORNA-21 AS A BIOMARKER IN LIVE ATTENUATED *LEISHMANIA* VACCINE INDUCED PROTECTIVE IMMUNITY

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No vaccine exists against visceral leishmaniasis. In an attempt to develop effective vaccines, we have reported extensively on the immunogenicity of live attenuated LdCentrin1-/- mutant in animal models. However, for the use of LdCen1-/- in humans there is a need to develop biomarkers associated with protection and safety. As a first step, we infected *ex*

vivo normal human macrophages with LdCen1-/- and compared with LdWT infection. We identified several microRNAs that regulate important cytokine genes, significantly induced upon LdWT infection compared to LdCen1-/- infection. Importantly, we found a strong induction of microRNA-21 (miR-21), which was shown to degrade mRNA encoding IL12, in LdWT infection compared to LdCen1-/- infection. IL12 produced by DCs is critical for priming a host protective Th1 cell response during *Leishmania* infection. To validate the role of miR-21 in regulating IL12 during *Leishmania* infection, we altered the miR-21 expression in murine DCs infected with LdWT or LdCen1-/-. Silencing of miR-21 using specific inhibitors resulted in an augmented induction of IL12 in LdWT infected BMDCs, illustrating the role of miR-21 in LdWT mediated suppression of IL12. In contrast, LdCen1-/- infected BMDCs, showed a strong induction of IL12, and miR-21 silencing resulted in a further increase in IL12 levels. Our data from *in vitro* human macrophages and mouse dendritic cell experiments suggests that miR-21 plays a role in early IL-12 mediated immunity and could be an important biomarker for LdCen1-/- vaccine immunity in human clinical trials.

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CTLA-4 AND ICOS COSTIMULATORS: POSSIBLE ROLE DURING ACTIVE VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is an endemic disease found in many countries around the world. In Latin America, *L.* is the main etiologic agent for VL. During symptomatic VL a temporary anergy, *Leishmania* antigen-specific, is observed, which is reversed after clinical cure. Coestimulation can be involved in anergy, when T lymphocytes tend to increase expression of molecules such as ICOS and CTLA-4. We evaluated those two costimulators in T lymphocytes within whole blood samples collected from subjects with symptomatic VL (sVL) and after their clinical recovery (rVL), in addition, cytokines were also measured. We found that during sVL there was an increase in CD4 and CD8 cells expressing CTLA-4, in *ex vivo* condition (exvc), when compared to rVL group ($p< 0.05$). Moreover, CD8 T cells from sVL expressed more CTLA-4 ($p< 0.01$) after stimulation by soluble *Leishmania* antigen (SLA), but not in rVL or control groups. A 9.3 fold increase in the relative expression of CTLA-4 was observed in sVL when compared to rVL; however, there was no difference when cells from the goups were stimulated with SLA (fold change of 0.702 and 0.992, respectively). An increase in CD4 and CD8 cells expressing ICOS, in exvc, when compared to rVL ($p< 0.01$ to CD4, and $p< 0.05$ to CD8) and control group ($p< 0.01$ to CD4 and CD8) was observed. After SLA stimulation, both CD4 and CD8 cells from sVL showed an increase in ICOS, when compared to unstimulated samples ($p< 0.01$ to CD4 and CD8). The relative expression of ICOS in sVL was 3.78 fold higher when compared with rVL; whereas, opposite results were found after SLA stimulation (2.8 fold change, and 0.5 fold change, respectively). The ratio of INFγ to IL10 was higher after clinical recovery. These findings support the role of CTLA-4 and ICOS in the reversible anergy observed during sVL and might indicate pathways to be explored for immunotherapy against visceral leishmaniasis.

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BIOMARKERS OF PROTECTIVE IMMUNITY INDUCED BY LIVE ATTENUATED *LEISHMANIA DONOVANI* PARASITES IN PRESENCE OF ASYMPTOMATIC INFECTION

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Currently there is no vaccine against visceral leishmaniasis (VL). Towards developing an effective vaccine, we have reported extensively on the immunogenicity of live attenuated LdCentrin1$^{-/-}$ mutants in animal models. In VL endemic areas, asymptomatic carriers outnumber symptomatic cases of VL (9:1) and are considered to be a reservoir of infection. Vaccination of asymptomatic cases represents a viable strategy to eliminate VL. Studies in our lab using LdCen1$^{-/-}$ parasites that secrete model epitopes have shown that experimental infection of mice with LdCen1$^{-/-}$ results in robust CD4+ memory T cell induction. Protection mediated by such immune memory against virulent challenge was observed in hosts that have not previously been exposed to infection. Immunological correlates of protection thus derived might have limited applicability in conditions where the immunized host has prior exposure to virulent infection. To examine whether LdCen1$^{-/-}$ parasites can induce protective immunity in experimental hosts that have low-level parasitemia from a previous exposure mimicking an asymptomatic condition, we infected mice with wild type *Leishmania donovani* parasites expressing LLO epitope 3 weeks prior to immunization with LdCen$^{-/-}$ parasites expressing 2W epitope to characterize the immune responses in the same host. Flow cytometric analysis of the antigen experienced T cells enriched using specific tetramers showed that comparable memory T cell responses (CD4$^+$ T central memory) represented by CD62L^{hi}, CCR7$^+$, and IL-7R$^+$ T cell populations can be induced with LdCen1$^{-/-}$ in asymptomatic hosts to that of LdCen1$^{-/-}$ immunization alone. These results demonstrate that LdCen1$^{-/-}$ immunization could be efficacious for use in asymptomatic VL individuals.

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B-CELL ACTIVATING FACTOR (BAFF) IS INVOLVED IN DEVELOPMENT OF SPLENOMEGALY DURING EXPERIMENTAL VISCERAL LEISHMANIASIS

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Splenomegaly is one of the major symptoms during visceral leishmaniasis (VL). However, the mechanisms underlying splenomegaly remain unclear. We previously reported that serum levels of B-cell activating factor (BAFF) in VL patients were significantly higher than those in healthy controls, as reported previously. Since mice overexpressing BAFF are known to show splenomegaly along with increased number of B cells, we examined if BAFF is also involved in splenomegaly during VL by using an experimental model. BALB/cA mice inoculated i.v. with 1×10^7 promastigotes of *Leishmania donovani* developed splenomegaly, with higher spleen weight at 12 and 24 weeks post infection compared with naive mice. Those infected mice with enlarged spleen had significantly higher levels of serum BAFF compared with naive mice. Flow cytometric analyses of splenocytes revealed increased CD19$^+$ (B cell marker) lymphocytes as a major contributor to splenomegaly in the infected mice. When BAFF gene knockout mice were infected with *L. donovani*, the spleen weights at 12 and 24 weeks of infection were significantly lower than those of infected wild-type mice. Increase of CD19$^+$ lymphocytes in the spleen after infection was significantly suppressed in BAFF-knockout mice compared with the wild-type mice. Taken together, these results suggest that BAFF-mediated increase of B cells is the major cause of splenomegaly during experimental VL.

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DETECTION OF A FLAGELLAR ANTIGEN OF *TRYPANOSOMA CRUZI* IN URINE OF PATIENTS WITH HIV/CHAGAS CO-INFECTION USING NANOPARTICLES

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is one of the major health problems affecting Latin American population. In patients co-infected with HIV,the reactivation of Chagas disease is almost always lethal and diagnostic tests are not effective in predicting reactivation ahead of time, when a pharmacological treatment would be life-saving. Currently immunological diagnosis based on the detection of anti-*T.cruzi* antibodies have poor specificity and sensitivity because it is affected by the genetic makeup of both the parasite and the human population studied.Antigen detection tests may provide a solution to these issues because they directly detect the presence of the parasite in a body fluid. Technical and biological issues that hampered antigen test development in the past include:low abundance,masking by high abundance resident proteins and extreme labiality. In order to address these issues we developed a affinity hydrogel nanoparticles that performs in one step a)concentration of the target analyte,b)size sieving and c) complete protection from degradation.This work aims to develop a novel test for the detection of *T.cruzi* antigens in urine. For this purpose we have developed new antibodies against flagellar protein of *T. cruzi*,from rabbits and chickens. The purified antibodies were evaluated by Western blot and were used in a magnetic ELISA assay to achieve a sensitivity of 0.5ng/mL. Affinity hydrogel nanoparticles will be used to increase the analytical sensitivity 100 fold and test diagnostic specificity and sensitivity in patients co-infected with HIV and Chagas disease.In parallel,an affinity hydrogel particles enhanced quantitative protein macro array test will be developed in order to verify the concentration of the *T. cruzi* flagellar antigen in the urine of patients. Our previous results using this system for spiked urine with flagellar protein of *T. cruzi* showed a sensitivity of 0.1ng. One goal of this work is to develop a self-working,low cost,visual urine test for Chagas disease that achieves a clinical sample sensitivity ten to 100 fold higher compared to existing technology.

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IMPROVING ACCESS TO ESSENTIAL OXYGEN THERAPY AND PULSE OXIMETRY FOR CHILDREN

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An estimated 15% of children under five who are hospitalized for pneumonia—the leading infectious cause of child mortality worldwide—have hypoxemia, and yearly around 1.5 million children with severe pneumonia require oxygen treatment. Hypoxemia is a risk factor for death in pneumonia—increasing mortality by five times in some settings—and is a complication of other common childhood diseases. Accurate identification of hypoxemia and provision of oxygen are essential components of a strategy to reduce child mortality, supported by new World Health Organization (WHO) guidelines on oxygen therapy in children and technical specifications for oxygen concentrators. Improvements in oxygen supply have been shown to reduce up to 35% of child pneumonia deaths. Despite its necessity for child survival, oxygen therapy is not prioritized nor included on the WHO or most national-level essential medicines lists for children, and oxygen supplies and pulse oximetry are often not available in many pediatric wards. We conducted stakeholder consultations with over 50 key informants to assess priorities and challenges associated with ensuring availability of oxygen and pulse oximetry in settings with a high burden of childhood pneumonia. Informants represented global and national policy, procurement, manufacturing, regulatory, and programmatic decision-makers. Key findings from the consultations and literature suggest policy leadership and financial investments are needed by governments, donors, and global manufacturers to increase availability of oxygen and pulse oximetry in low-resource settings. Identifying current coverage and barriers to access for oxygen are key to developing a full understanding of how to ensure inclusion of oxygen and pulse oximetry in current normative policies, treatment guidelines, health budgeting, system infrastructure, and programmatic priorities. Utilizing this evidence to advocate for prioritization of oxygen for child health and increasing availability of appropriate oxygen concentrators and pulse oximetry could help improve access to essential therapy and reduce pneumonia mortality in children.

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DRUG RESISTANCE AND MOLECULAR CHARACTERIZATION OF *MYCOBACTERIUM TUBERCULOSIS* ISOLATED FROM PULMONARY TUBERCULOSIS SUDANESE PATIENTS

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Currently, Sudan is suffering from many factors like tribal restlessness and poverty causing its population to be in a continuous movement in and outside the country in seek for a better life. These movements mostly lead to the transmission of diseases among which is pulmonary tuberculosis. WHO estimated that one third of the world's population are infected with *Mycobacterium tuberculosis* and approximately 9.7 million new cases of tuberculosis were diagnosed globally in 2013. Sudan is considered as one of the countries having a high prevalence of tuberculosis. The current study aimed at determining the susceptibility of *M. tuberculosis* isolates to the first line anti-tuberclosis drugs. Isolated organisms were also genetically identified to be allocated and compared with species identified elsewhere in the global. Two hundred forty three sputum samples were collected in the period from May 2007-October 2013 from central, eastern and western Sudan. These were namely; Khartoum state, Portsudan and Elobeid respectively. Sputa were examined microscopically after being fixed and stained with ZN stain. Sensitivity to Rifampicin; INH, Ethambutol and Streptomycin was tested using proportion method after establishing microbial culture. Moreover, Spoligotyping and MIRU-VNTR were used to discriminate between different strains of *M. tuberculosis* and results were compared to the international database. From this work it was obvious that tuberculosis is prevalent in different parts of Sudan and all age groups can are affected. 58% of the cultured samples were positive for *M. tuberculosis* in L.J. medium. The overall resistance to anti-tuberculosis drugs was 20% and Multi drug resistant strains (MDR) were 8.7% All strains were grouped into 28 different spoligotypes. A total of 70 strains have unique patterns and were considered as orphan strains. We recommend that further studies are to be done to identify other mycobacteria species causing TB and to investigate its association with drug resistance.

1769

THE USEFULNESS OF OXIMETRY IN TRIAGING FEBRILE CHILDREN AT OUTPATIENT LEVEL: EXPERIENCE FROM A CLINICAL TRIAL IN DAR ES SALAAM, TANZANIA

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The objective was to determine the usefulness of oximetry in the outpatient setting in detecting febrile children with severe respiratory distress requiring referral to a higher level of care. A sub-cohort of febrile children aged 2-59 months from a larger non-inferiority trial that investigates a novel electronic algorithm for management of fever in Dar es Salaam, Tanzania, was included. Oxygen measurement was performed in using a hand-held device in all children connected to the smartphone with the built-in algorithm. Children with cough and oxygen saturation of <90% received pre-referral antibiotic and salbutamol treatment and were referred to the nearest hospital. All children were followed until clinical cure or death. 1590 children were enrolled into this sub-cohort from December 2014 to February 2016 out of which 922 (58.0%) of patients had cough, 26 (2.6%) chest indrawing, and 20 (2.0%) a respiratory rate >97th %ile for age and temperature, respectively. 4 patients presented with hypoxemia (0.4%) of which all had other signs of respiratory distress, i.e. chest indrawing and respiratory rate >97th %ile for age and temperature. Two patients were eventually diagnosed with cyanotic congenital heart disease of which one patient died at the referral hospital, likely from incorrect administration of oxygen. The other two patients had lower respiratory tract infections and fully recovered. In conclusion, in the Tanzanian outpatient setting, hypoxemia is very uncommon and clinical signs and symptoms, including chest indrawing, may have superior performance in detecting children with severe respiratory distress requiring higher level of care. In resource-poor settings, oximetry and oxygen should be implemented in hospitals but might not be useful at peripheral level.

1770

EARLY TREATMENT OUTCOMES FOR THE FIRST COHORT OF PATIENTS INITIATED ON PULMONARY MULTI-DRUG RESISTANT TUBERCULOSIS TREATMENT AT PUBLIC REGIONAL REFERRAL HOSPITALS IN UGANDA

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Multi-drug resistant tuberculosis (MDR-TB) is an emerging public health concern in Uganda, with over 1,000 new cases notified in 2014. Before 2013, MDR-TB treatment was provided by Mulago National Referral Hospital (NRH) while Regional Referral Hospitals (RRH) only had the capacity to do surveillance for diagnosis and would refer patients to the NRH for treatment. However, patients diagnosed at RRH had challenges accessing the NRH. Since 2013, the USAID's SUSTAIN project has supported scale-up of provision of drug resistant (DR)-TB treatment at six Ugandan RRH using a combination of ambulatory and hospitalization models of care. We analyzed data on the first cohort of patients started on MDR-TB treatment at SUSTAIN-supported RRHs. This study was a retrospective descriptive analysis of data collected on 69 patients started on MDR-TB treatment at six RRHs between 1st April 2013 and 30th June 2014. Nineteen (30.4%) patients were female, 39 (56.5%) HIV-negative, 30 (43.5%) resistant to both isoniazid and rifampicin and 57 (82.6%) category 1 or 2 failures. At the start of MDR-TB treatment, their median age was 35 years (SD ±13.5), mean time-to-treatment initiation 96 days and out of the 30 HIV-positive patients, 27 (90%) were on anti-retroviral treatment with a mean CD4 count of 258. Within six months of treatment, fifty-nine (87%) patients achieved favourable treatment outcomes (45:65.2% culture converted at two months and 14:20.3% by the sixth month) while 10 did not (1: 1.5% did not culture convert while three: 4.4% each died, were transferred out, or were lost-to-follow up). During treatment, 32 (46%) patients experienced at least one severe drug adverse event and all were managed clinically. The median weight gain was 3.0 kilograms (SD ± 4.52). Despite delays in MDR-TB treatment initiation after diagnosis, a reasonably high proportion of patients achieved early culture conversion. Reasons for the high proportion of HIV-negative patients started on MDR-TB treatment should be investigated. These encouraging interim outcomes indicate a successful scale up of DR-TB treatment from NRH to RRH.

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EVALUATION OF VITAMIN D LEVELS AND PREVALENT TB AMONG HIV INFECTED IN ZAMBIA

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Vitamin D insufficiency is highly prevalent in the general population of the United States and appears to play an important role in immune regulation and tuberculosis (TB) progression. Little is known about Vitamin D in African HIV-infected populations. We thus evaluated the association of serum vitamin D levels with prevalent TB, incident TB, HIV progression, and biomarkers of nutritional status among new enrollees at an HIV clinic in Lusaka, Zambia at baseline and up to 12 months after enrollment. All consenting adults without current or recent history of TB were screened for TB regardless of symptoms according to current World Health Organization (WHO) recommended guidelines. Two sputa, one blood and one urine specimen were cultured. For participants consented to participate in a TB specimen repository for serum and urine specimens were stored at -80 oC. Baseline serum samples were analyzed for levels of Vitamin D, pre albumin, albumin, lipid profile, CD4+ count, viral load (VL), creatinine and hemoglobin. Vitamin D insufficiency was defined as <30 ng/ml, and deficiency <20 ng/ml. A total of 285 samples were tested. Forty-three (16%) patients were diagnosed with bacteriologically confirmed TB at enrollment. Mean age was 35 years; 47% were female; and mean body mass index was 22. Median CD4+ was 205 cells/mL and mean VL 4.6 Log10. Vitamin D insufficiency was detected in 43%(127/294) and deficiency in 28% (82/294). Patients with CD4<100 had higher pre-albumin, albumin and HDL-cholesterol (p<0.01), lower triglycerides (p<0.01), and were older (p,0.01)compared to other groups. No significant associations were found between baseline vitamin D levels and prevalent TB at enrollment or incident TB at 6 month. However, a trend was observed for baseline prealbumin levels to be lower in the prevalent (median 8.0 mg/dl) and incident (10.5 mg/dl) TB groups compared to participants without TB (16.0 mg/dl). High prevalence of Vitamin D insufficiency levels were observed in this population. Although not associated with prevalent or incident TB, further analysis is required to understand the immunological effect on HIV and or recurrent TB.

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IGG ANTIBODY SECRETION IN LYMPHOCYTE SUPERNATANT AMONG PAKISTANI CHILDREN WITH CONFIRMED TUBERCULOSIS

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Antibody Secreting Cells (ASCs) are terminally differentiated B-cells that release pathogen-specific antibodies in response to infection. ASC activity has been harnessed as a diagnostic test for infections including tuberculosis (TB): by isolating and culturing peripheral blood mononuclear cells (PBMCs) for 24-72 hours, the secreted anti-mycobacterial IgGs can be estimated via ELISA methodology using Bacille Calmette Guerin (BCG) vaccine as the coating antigen. Here, we aim to compare the yield of the MASC assay using different incubation time points among children confirmed to have pulmonary TB. Among a cohort of children 1-14 years old being evaluated for pulmonary TB, we included children with respiratory specimens that were positive for *Mycobacterium tuberculosis* (MTB) by GeneXpert. Venous blood (3-12mLs) was obtained for the MASC test. Briefly, mononuclear cells were cultured with 10% FBS at the concentration of 5-10x10^6 cells/ml in 24-well tissue culture plate at 37°C for 24, 48 or 72 hours. Culture supernatants were collected at each time point and stored at -80°C. ELISA plates were coated with 1µg / well of BCG vaccine (Japan BCG laboratory). Culture supernatants were added in ELISA plates after blocking nonspecific sites and incubated for 2 hours at 37°C. After washing, HRP labeled secondary antibody was added for the detection of IgG. The results were expressed as relative optical densities (O.D.) of IgG. A cut-off of 0.35 was used for a positive test [1]. Eight children with a median age of 12.5 years (IQR: 3.825) had GeneXpert positive respiratory samples. MASC results from six children were consistently above the threshold for a positive test at all incubation periods. The median ODs did not significantly differ across the selected incubation periods (p=0.727, Mann-Whitney U test; see Figure). Mycobacterium-specific IgG can be detected in lymphocyte supernatant from children with microbiologically confirmed TB. The assay demonstrates consistent results across the selected incubation periods, suggesting that this assay could be optimized to provide relatively rapid results, within 2-3 days after collection of blood.

1773

CHARACTERIZATION OF AN ALGORITHM FOR LOCAL SEASONAL INFECTIOUS DISEASE OUTBREAK DETECTION USING A SIMULATION STUDY

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Control of seasonal outbreaks plays a vital role in public health, particularly in healthcare settings where the young, elderly, and immunocompromised are at the highest risk. Early detection of seasonal virus outbreaks at the level of individual hospitals and community centers are key to controlling their epidemic potential. The Above Local Elevated Respiratory Illness Threshold (ALERT; http://reichlab.github.io/alert.html) algorithm is an online tool designed for clinicians and hospital epidemiologists to use in triggering enhanced protective measures for healthcare workers and hospital visitors. In this study, we use an influenza A dataset from a pediatric hospital to compare the date-based trigger strategy recommended by the CDC to the case-count threshold trigger implemented using ALERT. We found that a threshold case count of 3 was able to reliably out-perform the date-based threshold approach by reducing the median duration by 3 weeks, increasing the median percent cases captured from 93.5% to 95.1%, and increasing the minimum cases captured from 56.2% to 68.9%. Then we fitted a model to a subset of the flu A dataset and tested its ability to make one-step ahead predictions of the reserved testing subset of the flu A data. Once we confirmed that our model was representative of real seasonal influenza time-series, we used the model coefficients to generate data for a simulation study. We then systematically characterize ALERT performance on simulated data generated across a range of endemic and epidemic values in order to develop guidelines for when ALERT may be an appropriate tool for rule-based decision-making. We conclude with a discussion of these guidelines and suggestions for how ALERT may be leveraged for other infectious diseases, such as dengue fever, to enable clinicians and epidemiologists to make evidence-based public health decisions, particularly in low-resource settings.

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A GLOBAL FRAMEWORK FOR STRATEGIC TUBERCULOSIS PREVENTION AND CONTROL IN THE WORKPLACE

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Tuberculosis (TB) prevention and control activities in the workplace are an important consideration for multinational corporations with workforces in mixed-risk settings. Preventing TB transmission at ExxonMobil and its affiliated companies (EM) is especially challenging due to the global nature of our workforce and varied workplace settings which can include congregate living. Furthermore, EM operates in more than 50 countries, some having a high incidence of TB. To protect the workforce and minimize operation disruptions, EM has implemented a strategic TB Control Program to mitigate the risk of TB transmission among its workforce. Components of the program include worker awareness, conducting screening, coordination of care, and contact tracing. Eligible employees and contractors live in or travel to high-risk settings for 30 cumulative days per year. High-risk settings include all EM camps or dormitories in countries with TB incidence ≥ 20 cases per 100,000 population as well as all offshore sites. Workers in the program receive a baseline screening test upon enrollment, and are subsequently screened annually. Annual screening consists of a risk-based questionnaire and, when indicated, a T-Spot or Quantiferon screening test. For those testing positive, further evaluation is conducted for potential TB diagnosis and referral to a healthcare provider. If an active TB case is identified, contact tracing is promptly initiated in conjunction with relevant health authorities. Surveillance of cases through data management is key to stewardship of the program. In 2015, 9000 TB screening tests were performed, resulting in 1220 latent TB infections cases being identified among employees and contractors. Identification of these latent infections is imperative to effective TB control in the workplace, as early detection improves outcomes and overall productivity of the workforce, preventing illness and therefore potential for operations disruption. EM's TB Control Program is a pragmatic, tiered and scalable approach to mitigate the spread of TB among workers in areas of varying TB incidence, protecting our workplaces around the world.

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DECIPHERING LONG TERM DYNAMICS AND ASSESSING IMMUNIZATION CAMPAIGNS OF MEASLES IN CHINA

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Industrialization and demographic transition generate non-stationary dynamics in human populations that can affect the transmission and persistence of infectious diseases. Decades of increasing vaccination and development have led to dramatic declines in the global burden of measles, but the virus remains persistent in much of the world and measles is still one of the leading causes of vaccine-preventable childhood diseases. An international collaborative study was carried out by researchers from WHO, US and China CDC, and universities to decipher the dynamics of measles in China. Based on 50-year long term various measles surveillance data from national, provincial and city level sources, here we show that a combination of demographic transition, as a result of declining birth rates, and reduced prevalence, due to improved vaccination, has shifted the age distribution of susceptibility to measles throughout China. We estimate the relative change in the force of infection in 6 focal provinces across China as well as the impact of supplemental vaccination activities on the reduction of the susceptible population. The force of infection of measles has declined dramatically in the industrialized eastern provinces during the last decade, driving a concomitant increase in adult cases who had been protected from infection as children by herd immunity, while central and western provinces exhibit dynamics consistent with endemic persistence. The shift in the age distribution of susceptibility emphasizes the importance of progressive control strategies and measures to evaluate program success that anticipate this transition in observed incidence. Further, the regional differences in the persistence of measles across China suggest the importance of targeted efforts to interrupt transmission in endemic areas. We also developed novel modeling approach for immunization intervention effectiveness assessment when surveillance data is strongly biased or not available. The theoretical understanding and analytic approach in our study could shed light on how the ongoing global measles eradication campaign reaches its goal successfully.

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RESPIRATORY OUTBREAKS DURING AN OUTBREAK INVESTIGATION COURSE

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During a one-week outbreak investigation course in South America, the faculty noted an increase of respiratory symptoms among students and then conducted a quick outbreak investigation for academic purposes. The course has both a plenary component (25 hours) and case study component (11 hours) where the class is divided in six groups of 10-12 students. In course day 5, all 70 students were interviewed using a self-administrated questionnaire assessing signs and symptoms, demographic information, and location in the plenary and workgroup. Two case definitions were used, one based on self-report of "disease" and another using the most frequent signs and symptoms. Sixteen students (23%) reported themselves as "sick", 63% with headache, 56% with sneezing, 56% with rhinorrhea, 31% with sore throat, 31% with cough and 25% with fever. Up to 3 cases started symptoms on each of course days 1-4 and seven started symptoms in day five. The attack rate differed significantly by case study groups (range: 0-75%, $p<0.001$) and also by plenary table row ($p=0.012$) but not by plenary table column, gender, age or region of origin. An alternative, parsimonious case definition of sneezing (24 cases) or headache (12 cases), led to an attack rate of 29 cases (41%), capturing 15 cases not self-identified as "sick" and all but two cases with any symptoms. Most new cases (87%) only reported sneezing. Incidence of cases found only with the second definition differed by case study group (0-60%, $p=0.001$) but not by other characteristics. The case study groups with highest incidence differed for self-defined and not self-defined cases. A similar course was taught later in Central America without noticing potential outbreaks of respiratory illness. Two clearly-differentiated outbreaks may have presented in a five-day course, suggesting person-to person-transmissions of separate respiratory viruses, both with short incubation period probably due to close proximity of students, particularly during case study groups.

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IMPLICATION OF SOUND RECORDING SYSTEM ON TREATMENT SUCCESS FOR TB PATIENTS IN PORT HARCOURT NIGERIA

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In 2006, the World Health Organisation (WHO) established a Global Task Force on tuberculosis (TB) Impact Measurement and one of the mandates was to strengthen national capacity in monitoring and evaluation. This requires improvement of data quality especially completeness and accuracy of records. In Nigeria as in most countries, each TB patient has a treatment card on which the most relevant processes for patient care are documented. Proper completion of all of these care processes in the patients' treatment card is essential to survey the disease dynamics, assess program progress/gaps and plan for future interventions. The aim of this study was to determine the completeness of TB patients' records

and its implication on treatment outcome. This was a facility-based cross-sectional study, using data from treatment cards of 243 patients seen from November 2012 to October 2013. Following data extraction, proportions were calculated for completeness of patient treatment cards. Chi-squared statistic was computed for dependent variables such as treatment success and independent variables such as sputum AFB tests. Logistic regression was done to determine predictors of treatment success. Of the 243 patient treatment cards reviewed, only 23.9% were complete. Assessment of the individual variables revealed the following proportions of completeness: initial AFB test - 84.8%; 2nd AFB - 74.1%; 3rd AFB - 47.6%; weight at commencement - 99.2%; 2nd weighing - 72.8%; 3rd weighing - 46.5%; intensive phase treatment - 99.2%; continuation phase treatment - 77%. The treatment success was 49%. Predictors of this outcome were: complete acid alcohol fast bacilli tests, odds ratio 5.18 and 95%CI (2.08 - 12.89); and compliance to continuation phase, odds ratio 8.47 and 95%CI (3.31 - 21.68). The WHO targets for STOP TB are dependent on the credibility of readily available data generated from the health facilities. The completeness of records in the facilities assessed for this study adversely affects the validity of the treatment outcomes recorded.

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ANTIMYCOBACTERIAL AND PHYTOCHEMICAL ANALYSIS OF METHYL TERT-BUTYL ETHER EXTRACTS FROM THE FRUIT SKIN AND LEAVES OF *ANNONA MURICATA* LINN

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Arising from the Millennium development goals and Stop TB strategy of the World Health organization into the Sustainable goals cum End TB strategy, there is urgent need to fast-track research along proffering solution to the agelong burden of Tuberculosis. The current study examined the antimycobacterial activity and phytochemical constituents of methyl tert-butyl ether (MTBE) extracts from the fruit skin (epicarp) and leaves of *Annona muricata* Linn. The extracts were prepared from the matured unripe fruits and leaves of *A. muricata* with MTBE for accurate lipidome profiling. Antimycobacterial activity was determined Drug susceptibility testing (DST) procedure on Lowenstein Jensen (LJ) media. Three concentrations (1, 40 and 250 µg/ml) of the extracts were prepared with the LJ media and subsequently inoculated with 10-3 and 10-5 suspensions of both control (H37Rv) strain and a clinical isolate (MTB-584) of Mycobacterium tuberculosis. LJ media prepared with Rifampicin at 40 µg/ml served as the standard drug for positive control while plain media with respective inoculum represented the negative control. Four Ziehl-Neelsen's stain slides were also prepared to confirm the presence of organisms in the two suspensions employed for the two strains tested. Plain media with drops of distilled water were employed as normal control to check for possible contaminant. The inoculated media and control slants were incubated at 37oC and observed every seven days for a period of six weeks. The antimycobacterial analysis result showed that the organism strains exhibited resistance to the extracts at tested concentrations as there was substantial growth with typical creamy non-pigmented morphology on all the LJ media prepared with extracts. There was no growth on the media with standard drug and on those with distilled water as expected. Tannins, saponins, flavonoids, anto- and betacyanins, terpenoids, phenols and steroids were present in the extract. The conclusion from the foregoing is that MTBE extracts from the fruit skin and leaf of *A. muricata* at tested concentrations have no antimycobaterial activity.

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A LECTIN-BASED ASSAY FOR DETECTION OF SCHISTOSOMIASIS

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Schistosomiasis is a debilitating neglected tropical disease caused by trematodes of the genus *Schistosoma*. Current serological tools based on antibody detection lack the capacity to distinguish current from former infections after successful chemotherapy. A commercially available point-of-contact test to detect adult worm circulating cathodic antigen (CCA) in urine has been developed and is being evaluated for use in control programs. However, this test only reliably detects *S. mansoni* infections and may therefore not be as useful in *S. haematobium*-endemic areas in Africa. Furthermore, it has been difficult to conclusively determine whether persons who are CCA+ but *S. mansoni* egg negative are truly infected or may have non-patent infections. Another adult antigen, circulating anodic antigen (CAA) is more sensitive than the CCA test and can detect both *S. mansoni* and S. hematobium infections. However, this optimal sensitivity of this test requires a concentration step and is not currently amenable to point-of-contact testing. Both the CCA and CAA tests use monoclonal antibodies for antigen capture and detection, which adds to the cost of the tests. Therefore, although the CCA and CAA tests show promise for use in schistosomiasis control programs, we were also interested to see if it would be possible to detect free glycans secreted from eggs of both *S. mansoni* and S. hematobium. Schistosome-specific glycans terminating with α (1-2) fucose attached to internal N-acetyl glycosamine and N-acetyl galactosamine were investigated as potential diagnostic targets for schistosomiasis. Lectins from Ulex aeropaus and wheat germ agglutinin that bind these glycans with high specificity were functionalized with gold nano particles, agarose beads or horseradish peroxidase and used to detect schistosome egg glycans in lateral flow and ELISA assays. Urine from persons with *S. mansoni* and S. hematobium infections were positive by this test but urines from persons living in non-endemic areas were not. These results show promise for development of an inexpensive point-of-contact assay that is able to detect patent *S. mansoni* and S. hematobium infections.

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THE PERSISTENT PARASITE: WHY DO *SCHISTOSOMA MANSONI* INFECTION LEVELS REMAIN HIGH IN THE RURAL UGANDAN VILLAGE OF WAKAWAKA EVEN AFTER OVER A DECADE OF TREATMENT?

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Despite more than a decade of mass preventive chemotherapy, age-infection profiles of *Schistosoma mansoni* have shown that prevalence and intensity of infection in Eastern Uganda are still high. With a global shift to the elimination of schistosomiasis in the 2012 World Health Assembly resolution, there is a need to identify why more than 70% prevalence and high intensities of infection are still identified in those most at-risk populations, school-aged children. The aim of this study was to identify possible non-biological contributors to this trend by interviewing 248 individuals across all age groups in Wakawaka village, a large fishing community, in the district of Bugiri. The survey explored the social, behavioural and economic background of the participants, in addition to gathering information on living environments and access to healthcare. For children aged between 3 and 15 years an additional innovative activity was carried out which involved a colouring sheet identifying transmission routes. Only half of the colouring activity questions were marked correctly. For the survey, results revealed 80% of the participants continue to

use Lake Victoria as their primary water source for activities other than drinking. Additionally 67% thought that most 'worm based infections' came from drinking dirty water and only 18% of participants correctly identified that swimming in contaminated water was a source of infection. Knowledge of STH were similar with 98% unable to identify walking barefoot and not washing hands as risk behaviours and only 30% of the survey participants forbidding their children from defecating in the open. Almost 14% of the population had lived in the area for 2 years or less. Results show that there is still a dearth of community level understanding of schistosomiasis and STH and their transmission routes despite numerous treatment rounds and high levels of infection. If programmes are to move from control to elimination, then we need to strengthen current strategies with improved treatment coverage and sensitisation, taking into account communities that are mobile, access to safe clean water and community awareness.

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COMMUNITY-WIDE PATTERNS OF INFECTION AFTER MORE THAN TEN YEARS OF PREVENTIVE CHEMOTHERAPY FOR SCHISTOSOMIASIS AND SOIL-TRANSMITTED HELMINTH INFECTION IN UGANDA: ARE WE READY TO MOVE BEYOND MORBIDITY CONTROL?

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In 2003, Uganda was one of the first sub-Saharan African countries to implement national-scale control programmes for schistosomiasis and soil-transmitted helminth (STH) infection, which have aimed to control infection-associated morbidity through mass drug administration (MDA) of suitable anthelmintics to school aged children (SAC) and other high risk groups. SAC harbour the highest prevalence and intensity of schistosome infections which then typically decrease with age. Control programmes often incorporate a monitoring and evaluation (M&E) component to estimate a programme's impact on population levels of infection, using data collected from SAC. More data are needed, however, to understand the impact on the wider community when only SAC are targeted; this understanding is essential to determine the feasibility of shifting the focus towards elimination. Using baseline data from 2003 and data from a two year full age-infection (AI) survey from 2014 and 2015, we aim to provide an accurate picture of the change in infection patterns in Uganda. In the full AI study, data were collected from approximately 7500 individuals each year, across a wide age-range (<1 to >50 years) from 10 different sites in Uganda, which varied by initial underlying endemicity and treatment history. Results showed that the AI profiles for *Schistosoma mansoni* and hookworm followed similar patterns as observed at baseline and in other studies. Between 2014 and 2015, no significant treatment impact was observed for *S. mansoni* and hookworm. When analysing results by underlying endemicities, the high prevalence sites and the low prevalence sites, both of which had received multiple rounds of treatment since 2003, showed no reduction between the two years. Conversely, for the low prevalence sites that had not received treatment until 2014 (i.e. previously treatment-naïve), we observed a decrease in prevalence and intensity. We will discuss the age-related changes in intensity and prevalence in each subset; possible explanations for the trends observed and their significance levels; and compare the age-specific force of infection from baseline to the recent data.

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MODELLING THE EFFECT OF A POTENTIAL VACCINE APPLICATION ON THE SCHISTOSOME PARASITE DYNAMICS

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Schistosomiasis affects approximately 258 million people, killing an estimate of 280 thousands per year. This makes the development of an effective vaccine to help, alongside mass drug administration (MDA), in the elimination of the disease within the near future a necessity. To date, the primary form of treatment is the drug praziquantel, which gives some reductions in the burden of the infection but repeated annual administration is required over many decades to eliminate the burden of disease. A potential vaccine candidate could act to reduce parasite establishment, survival and fecundity within the host population. Analysed data from experiments with a candidate prophylactic vaccine application in a nonhuman primate model, the baboon, gives evidence that the development of a partial efficacious vaccine may be a possibility. We describe the construction and use of mathematical models of candidate vaccine community based impact alongside the use of MDA. We focus on vaccination effect on both the host population and the parasite's dynamics. We run the models under different scenarios by taking into account various crucial assumptions about the vaccine candidate. These include the effectiveness of the vaccine and the rate of loss of vaccine-induced immunity. We also run the simulated models for the combination of mass drug administration (MDA) and community based cohort vaccination to test if the elimination of the disease can be achieved more quickly with a partially efficacious vaccine.

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UROGENITAL SCHISTOSOMIASIS: WHAT DO SCHOOLCHILDREN IN THE EASTERN REGION OF GHANA KNOW ABOUT THE DISEASE?

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Urogenital schistosomiasis (UGS) is endemic in the Eastern Region of Ghana, particularly amongst primary-school aged children in rural communities. Educating children about UGS through the public school system in endemic areas can be an effective primary prevention strategy that accompanies mass drug administration. However, little is known about the baseline knowledge of schoolchildren in the Eastern Region of Ghana regarding UGS, and the individual and community-wide characteristics that predict levels of knowledge. The objective of this study was to determine the baseline knowledge of students in the Eastern Region of Ghana regarding UGS, and then to determine the extent to which year in school, sex, and district of residence predict UGS knowledge among schoolchildren. Over a 17-day period, we conducted a cross-sectional study among 1813 primary and junior high school children in public schools across 37 randomly selected towns within 10 districts in the Eastern Region of Ghana. All participants were given a 22-question knowledge survey on the transmission, treatment, and symptoms of schistosomiasis and protective measures that can be taken to prevent infection. A score was assigned to each student representing the number and percentage of questions answered correctly. Overall, the average score on the knowledge survey was 57.5%. Junior high school students had a mean score of 63.0% while primary school students had a mean score of 51.5%. Responses indicate that knowledge of how the disease is transmitted and how the disease can be treated is lacking among both primary and junior high school students. Linear regression analyses indicate that sex, class year, and district of residence are predictive of student performance on the knowledge survey, with class year as the strongest predictor. Linear regression and chi-squared analyses indicate that boys systematically perform better than girls on the knowledge survey, and

junior high school students systematically perform better than primary students. These findings are valuable for officials engaged in the planning and implementation of UGS educational interventions.

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SPATIOTEMPORAL MODELING OF SCHISTOSOMIASIS IN GHANA: LINKING REMOTE SENSING DATA TO INFECTIOUS DISEASE

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More than 90% of the worldwide schistosomiasis burden falls on sub-Saharan Africa. Control efforts are often based on infrequent, small-scale health surveys, which are expensive and logistically difficult to conduct. The use of satellite imagery to predictively model infectious disease transmission has great potential for public health applications. The transmission of schistosomiasis, a disease acquired from contact with contaminated surface water, requires specific environmental conditions to sustain freshwater snails. If a connection between schistosomiasis and remotely sensed environmental variables can be established, then cost effective and current disease risk predictions can be made available. Schistosomiasis transmission has unknown seasonality, and the disease is difficult to study due to a long lag between infection and clinical symptoms. To overcome these challenges, we employed a comprehensive 15-year time-series built from remote sensing feeds, which is the longest environmental dataset to be used in the application of remote sensing to schistosomiasis. The following environmental variables will be used in the model: accumulated precipitation, land surface temperature, vegetative growth indices, and climate zones created from a novel climate regionalization technique. This technique, improves upon the conventional Köppen-Geiger method, which has been the primary climate classification system in use the past 100 years. These predictor variables will be regressed against 8 years of national health data in Ghana, the largest health dataset of its kind to be used in this context, and acquired from freely available satellite imagery data. A benefit of remote sensing processing is that it only requires training and time in terms of resources. The results of a fixed effects model can be used to develop a decision support framework to design treatment schemes and direct scarce resources to areas with the highest risk of infection. This framework can be applied to diseases sensitive to climate or to locations where remote sensing would be better suited than health surveys.

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EPIDEMIOLOGICAL MAPPING OF SCHISTOSOMIASIS AND SOIL TRANSMITTED HELMINTHIASIS IN 19 STATES AND THE FEDERAL CAPITAL TERRITORY (FCT), NIGERIA

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The coordinated mapping of Schistosomiasis and Soil Transmitted Helminthiasis (STHs) was conducted in 19 States and the FCT of Nigeria from November 2013 to May 2015.Both diseases were mapped using a novel technique; the LINKS system developed by the Task Force for Global Health (TFGH), on Android devices and cloud based data reporting and management. The application of these devices supported the transition from paper-based questionnaires to electronic data collection tools. A total of 108,472 children from 2160 schools in 433 LGAs in 19 States and the Federal Capital Territory (FCT) were examined for Schistosomiasis and STHs. The Kato-Katz, filtration techniques were used to examine stool and urine samples Also, Water, Sanitation and Hygiene (WASH) information for schools and school children were collected. The result of this survey revealed that all the States and the FCT were endemic for one or both diseases with an overall prevalence of 9.5% for Schistosomiasis and 27% for STHs. However, the data captured by LGA; the intervention unit, showed that prevalence of infections varied from low to high risk. The prevalence of infections was significantly higher in males than in females for both diseases. STHs were more prevalent among the younger age group (5-10years) while Schistosomiasis was more prevalent among the older age group (11-16 years).Heavy intensity levels were nearly equal for S.haematobium (24.31%) and *S. mansoni* (23.48%). The intensity levels of *S. haematobium* and A. lumbricoides showed statistical significant difference ($P<0.05$) with respect to sex in this survey. STHs and Schistosomiasis were seen among pupils who claimed to defeacate in the school toilets, around the school compound and outside school environment. Of the 433 LGAs surveyed the number of LGAs requiring interventions for Schistosomiasis and STHs were 359 and 237 respectively. The mapping exercise provided insight into disease distribution and intensity in 19 States and the FCT. It is recommended that Government and stakeholders should scale up mass deworming alongside WASH interventions.

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EVALUATION OF A URINE POOLING STRATEGY FOR THE RAPID AND COST-EFFICIENT PREVALENCE CLASSIFICATION OF SCHISTOSOMIASIS

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Schistosomiasis is geographically focal, making it difficult to target with mass treatment through preventive chemotherapy. The aim of the present study was to evaluate the diagnostic performance of a urine pooling strategy using a *Schistosoma mansoni* point-of-care circulating cathodic antigen (CCA) test, and then use simulation modeling to test the classification accuracy and efficiency in determining where preventive chemotherapy is needed in low burden settings. We performed a cross-sectional study in 114 children in six neighborhoods in Azaguié Ahoua, Côte d'Ivoire to characterize the sensitivity and specificity of the CCA test with urine samples that were tested individually and in pools of 4, 8, and 12. We used a latent class model to estimate test characteristics for individual CCA and quadruple Kato-Katz thick smears. We then developed a microsimulation model and used Lot Quality Assurance Sampling to test the performance of the urine pooling strategy and traditional stool microscopy in predicting the binary need for school-based preventive chemotherapy using WHO's 10% prevalence threshold recommendation. We estimated the number of tests and total cost of each strategy, and tested robustness of the simulation through sensitivity analyses. The overall sensitivity of the urine pooling strategy for pool sizes of 4, 8, and 12 was 85.9%, 79.5%, and 65.4% when CCA trace results were counted as positive, and 61.5%, 47.4%, and 30.8% when CCA trace results were counted as negative. The modeled specificity ranged from 94.0-97.7% for the urine pooling strategies (with trace CCA results

categorized as negative). The urine pooling strategies (pool size=4, 8, 12) gave comparable, and often superior, classification performance to stool microscopy for the same number of tests, and the urine pooling strategy (pool size=4) reduced number of tests and total cost compared to stool microscopy. This study introduces a rapid, cost-saving urine pooling strategy to inform where preventive chemotherapy against intestinal schistosomiasis is necessary that does not depend upon slide preparation, microscopy, or a formal laboratory.

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SIZE MATTERS: CHANGING POPULATION STRUCTURE MEANS CHANGING SAMPLING REQUIREMENTS FOR SCHISTOSOME POPULATIONS

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Eradication or local extinction of schistosomiasis is a goal for nearly all control programs today. We have demonstrated how genetic markers can be used to evaluate control programs, indicate incipient resistance and perhaps predict the extinction of a local population. Our studies have been conducted by collecting parasites from all identified infections in a population to calculate individual host differentiation indices (Di) and community effective population size (Ne). Collecting all individuals and genotyping their parasites, however, would be impractical on a large-scale, thus we sought to determine the relative error associated with different sample sizes. Using data collected in 2009 and 2012 from two villages in Bahia, Brazil, we calculated Di based on samples of different sizes. Individual infections were selected at random with replacement to produce samples ranging in size from 5-50% of the total. Each size class was repeated X 30. Di was calculated for each using SPADE. Error rates of ± 5%-10% of the true value of Di were taken as acceptable limits. The percent of groups outside this range was then calculated. We thus compared 2009 and 2012 for these communities, since the Di and Ne changed following community-wide treatment. Between 2009-2012 there was no difference in Di for one, but did increase for the other. Ne fell by 15 fold for each site. When the Di is moderate and Ne large, taking samples of 30-40% of the population was within the 10% limit 60% of the time. When the Di increased and Ne reduced, the curves were less steep, but shifted upward so that samples from composed of 30% of the infected had only a 50% chance of being within 10% of the true value and where the Di was significantly higher, there was only a 40% chance of being in this range. The chance of obtaining differentiation indices outside of the acceptable error range with smaller sample sizes increases when the population has undergone a bottleneck. In order to acquire the most representative results for population genetics of *S. mansoni* some characteristics such as population size, prevalence of the parasite, history of treatment in the community has be taken into account.

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SCHISTOSOMIASIS DIAGNOSIS AND CLINICAL MANAGEMENT: USE OF IMMUNODIGNOSIS, DNA BASED ASSY AND DETECTION OF CIRCULATING CATHODIC ANTIGEN (POC-CCA) PRE AND POST-PRAZIQUANTEL IN NON ENDEMIC AREAS

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Diagnosis of *Schistosoma mansoni* infection in travelers and immigrants living in nonendemic areas can be troublesome. Parasitological methods and tissue biopsy underdiagnosis acute and chronic infection with no or low egg counts. As an alternative to traditional methods, rapid tests (RT) like POC-CCA has been used in some institutional settings. However, no consensus toward the use of RT in clinical protocols was achieved. The study objective was to evaluate POC-CCA in both schistosomiasis diagnosis and post-therapy response in non-endemic areas. Thirteen individuals living in the non-endemic area with a history of previous exposure to S.mansoni participated in the study. Fecal samples were tested by Kato-Katz (KK) and DNA amplification by Real-Time PCR. Tissue biopsy was also performed when available. Serum IgG1 anti-adult worm (SMMA) levels were detected by ELISA (arbitrary units:a.u., positive > 1 a.u.). For CCA detection, urine samples were tested by POC-CCA (Rapid Medical Diagnostics, Pretoria, South Africa). Praziquantel (40mg/kg) was used for treatment. Responders to therapy were defined as KK and/or PCR negative (KK/PCR). Nine male and four female (mean age:34.5±15.9 years old) participated in the study. Seven individuals presented asymptomatic infection and two manifested acute schistosomiasis. Intestinal severe form and neuroschistosomiasis were diagnosed in two and one individual, respectively. Active schistosomiasis was confirmed by KK in 7/13 individuals, being 6/13 KK negative. IgG levels detected 10/13 and reactivity varied from 0.1 to 4.9 a.u.(mean±std: 1.7 ± 1.19 a.u.). Real-time PCR showed DNA amplification in 13/13 individuals (Ct mean ± std:28.03 ± 14.09). POC-CCA was reactive in 9/13 individuals (mostly weak reactivity or trace). After PZQ treatment, cure rates determined by KK/PCR and POC-CCA were 100% and 61.53%, respectively. KK/PCR combined and POC-CCA is a reliable diagnostic strategy to detect active *Schistosoma* infection in nonendemic areas. However, use of POC-CCA as a marker of drug response is still debatable.

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SCHISTOSOMIASIS IN SUB-SAHARAN AFRICA: SUCCESSES AND BARRIERS TO COMPLETE ERADICATION

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From the narrative of the German explorer of Central and West Africa, Gustav Nachtigal, Schistosomiasis, a neglected tropical disease of poverty has plagued various nations in sub-Saharan Africa since 1881. Schistosomiasis exert great health, social and financial burden on the economies of the region having profound negative effects on child development, outcome of pregnancy, and agricultural productivity. Sixty-seven percent (11.7 million) of people treated in 2008 for schistosomiasis, were from sub-Saharan Africa with Nigeria being the most endemic country for schistosomiasis, burdened by approximately 20 million people mostly children needing treatment. While countries such as Japan, Tunisia and some Caribbean Island countries have made significant progress on the control and management of this disease, sub Saharan countries are still groaning under the burden of this impoverishing disease. This review focuses on the history, epidemiology, successes, and barriers impeding the complete eradication despite significant re-awakening efforts by such organizations as, WHO, State Ministries of Health and the Carter Center to end the anguish of this silent disease.

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THE ASSOCIATION OF RESISTANCE TO *SCHISTOSOMA MANSONI* REINFECTION AND HOST IMMUNITY IN MBITA KENYA COHORT

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Schistosomiasis has been a threat for inhabitant living in endemic area where it is inevitable to contact with the schistosome cercaria infested water in daily activities. Without treatment, schistosomiasis can persist for years and develop to chronic infection with egg-induced pathologic complications including liver fibrosis, bladder fibrosis and cancer. Identification of protective immunity against schistosomiasis is really critical for strategy of vaccine development. In Mbita, Kenya, a *Schistosoma*

mansoni endemic area, we enrolled 160 preschool- and school-age children aged 4-15 years and monitored them by Kato-Katz examination and circulating cathodic antigen (CCA) tests. The children with egg positive stools or CCA positive urine were cured twice with Praziquantel and 2-month interval. Three months after the last treatment, the examination showed that 20% (32 cases) were re-infected (susceptible) with egg positive stools, 55% (88 cases) non-infected (resistant) with egg negative stools, 7% (11) with natural non-infection and 18% (29) did not supply stool samples. Immunological investigation indicated that plasma levels of SWA-specific IgG1 was higher in resistant- than susceptible-children (absorbance of 450nm: 0.271 vs 0.135, $p < 0.05$, respectively) whereas there was no difference in plasma levels of SWA-specific IgG4. Production of IFN-γand IL-13 from blood cells under stimulation of SWA were higher in resistant- than susceptible- children (793 vs 15.6 pg/ml; 272 vs 68 pg/ml, respectively, $p < 0.05$). Meanwhile, IL-10 production was similar in these two groups of children. Under non-specific stimulation, resistant children possess higher proportions of IFN-γproducing CD4+ T and non-CD4 non-CD8 T cell than susceptible children (2.2% vs 1.19 %; 0.76% vs 0.34%, respectively, $p < 0.05$), whereas there was no difference in proportion of IL-13 producing CD4+T cells in these two groups (1.58% vs 1.78%, respectively). In conclusion, anti-SWA IgG1 antibody, CD4+ and non-CD4 non-CD8 cell derived IFN-γ and IL-13 could be protective factors against reinfection of S.mansoni.

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DEMOGRAPHIC COVARIATES OF CHOLERA RISK IN CAMEROON

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Since cholera's arrival in Cameroon in 1971, the country has experienced multiple outbreaks with thousands of reported cases. The outbreak in 2010-2011 has been Cameroon's largest, with over 27,000 people infected. During the 2010-2011 outbreak, the cholera attack rates among Cameroon's 179 health districts varied widely, ranging from 0 to 1,139 cases per 100,000 people. Using data compiled in the Demographic and Health Surveys and national surveillance data reported by the Ministry of Health, this study examines the relationship between demographic covariates and cholera risk. In a country-wide analysis of all health districts, we found the number of children under five living in a home and family size to be positively associated with attack rate. When analyzing health districts outside of the southwestern part of the country, higher education, access to improved sanitation, and higher SES were all negatively associated with cholera attack rates. Access to improved water and cholera attack rates were not associated. In the southwest part of the country, the covariates were no longer sensible predictors of cholera risk. Different environmental conditions in the southwestern part of Cameroon may be driving these results, for example as a consequence of the construction of the Bamendjin dam. The results suggest that basic demographic variables may serve as useful predictors of cholera risk and, in conjunction with environmental variables, may inform policy including the deployment of the oral cholera vaccine stockpile.

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SHARED SANITATION FACILITIES AND TWO PATHWAYS OF DIARRHEAL DISEASE TRANSMISSION: A MODELING STUDY

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In many low and lower-middle income countries, access to sanitation is limited for many individuals. Open defecation contaminates the environment and facilitates the transmission of diarrheal pathogens that are transmitted via the environment. The provision of sanitation facilities that are shared by many individuals, such as shared latrines in slums, are considered by many public health officials as the only short-term solution to this problem, especially in urban slums in Asia and Africa. However, epidemiological evidences have shown that shared sanitation may actually increase the prevalence of diarrheal diseases. One hypothesis that may explain this phenomenon is that many different pathogens may cause diarrhea. While sanitation facilities reduce the contamination of the environment by human defecation, unhygienic sanitation facilities are actually fomites for the transmission of other diarrheal pathogens that are transmitted directly between humans. We propose a mathematical model that seeks to explain how the alleviation of environmental transmission of pathogens such as cholera via shared sanitation can lead to an amplification of direct transmission caused by other diarrheal diseases such as norovirus. The model is an ordinary differential equation (ODE) model that runs parameters chosen from a Latin hypercube sampling. After sieving through the parameter space to select parameter sets yielding outbreaks within a specified threshold, varying levels of shared sanitation coverage are implemented on these sets. Changes in disease prevalence are then plotted with respect to these varying levels of shared sanitation intervention. Results give quantifiable evidence that in the presence of an environmental and a person-to-person spread pathogen, total disease prevalence can increase. Under certain conditions the model shows an optimal level of shared sanitation intervention that can decrease environmental disease prevalence while not increasing person-to-person transmission too much. In any case, shared sanitation is most effective when viewed as a long term strategy.

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NOT IN MY BACKYARD: AN INDIVIDUAL-LEVEL META-ANALYSIS OF THE ASSOCIATION BETWEEN COMMUNITY OPEN DEFECATION AND STUNTING

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Approximately 1 billion people live without access to proper toilets and practice open defecation, a practice which the Sustainable Development Goals wishes to end by 2030. Open defecation facilitates the transmission of various diarrheal diseases as well as soil-transmitted helminthes. Access to sanitation decreases the risk of these diseases and any subsequent issues with development thereafter, including stunting. We conducted an individual-level meta-analysis of 177 publicly available, nationally representative household surveys to measure the impact that living in an open defecation free community has on child growth stunting among children aged 12-59 months. To account for selection bias we first matched children on the following parameters: community-level wealth, individual-level wealth, community-level water access, community-level health care access, and mother's education. Second we stratified the analysis among children living in households with and without a latrine. We then adjusted for a variety of factors known to be associated with child stunting (i.e. breastfeeding, immunizations, birth order, wealth, drinking water) and measured the incremental impact of community-level latrine ownership among children with and without latrines. Among children living in households with latrines, living in open defecation free communities (all households have latrines) was associated with decreased odds of stunting (odds ratio [OR] = 0.95, 95% confidence interval [CI] = 0.92 - 0.99). Among children living in households without latrines, living in communities with less open defecation was associated with decreased odds of stunting (OR = 0.96, CI = 0.93 - 0.99). The elimination of open defecation is an important sustainable development goal and as the results of our study suggests can subsequently have important benefits in health as indicated by child stunting.

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COMMUNITY BASED METHODS FOR SCHISTOSOMIASIS PREDICTION AND SUSTAINABLE CONTROL

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Schistosoma haematobium transmission is influenced by environmental conditions that determine the suitability of the parasite and intermediate host snail habitats, as well as by socioeconomic conditions, access to water and sanitation infrastructure, and human behaviors. We present a mixed-methods approach that builds on the remotely sensed ecological variables by exploring water and sanitation related community characteristics as independent risk factors of schistosomiasis transmission. The study area includes 74 rural communities in the Eastern Region of Ghana. Environmental conditions relevant for disease transmission such as stagnant or slow moving water bodies, riparian vegetation and water surface temperatures have been derived using remote sensing data from the Landsat 8 and Sentinel 2A satellites, as well as the 30m Advanced Spaceborne Thermal Emission and Reflection Radiometer Global Digital Elevation Model (ASTER GDEM) and integrated into a habitat suitability index (HSI). Additionally, for each study town, GPS coordinates and basic field survey data were available for all public water sources including improved water infrastructure according to the Joint Monitoring Program definition, and surface water access points. We calculated improved water coverage expressed as % of population with access to an improved water source within 100-500 meters of residence and groundwater quality score related to iron, salinity and hardness of the water as well as a recreation potential score. The HSI was complemented with community specific variables to predict schistosomiasis risk based on the hypothesis that in a small geographical area with minimal variability in environmental conditions, these potential community level drivers of surface water contact increase schistosomiasis risk. We validated the model using schistosomiasis prevalence data from a field survey.

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THE ROLE OF ENVIRONMENTAL PROCESSES IN INFECTIOUS DISEASE DYNAMICS

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For many infectious pathogens, the environment plays an important role in transmission from one host to another. This environmental mediation may occur through a number of media—including air, food, and fomites—but water is especially of interest in the transmission, fate, and transport of enteric pathogens. Mitigation, therefore, often involves environmental WASH interventions designed to reduce one's exposure to pathogens. Mathematical modeling can be used as a tool to investigate and assess potential interventions, allowing for more effective intervention design and allocation of resources, and has been used in the past to study waterborne outbreaks, notably in the recent cholera epidemic in Haiti. We leverage modeling of dose-response relationships and pathogen persistence in the environment to provide an improved mechanistic understanding of interventions. We conduct a comparative analysis to assess interventions that are designed explicitly to reduce per-contact pathogen load—such as water filtration devices—and those designed to reduce the frequency of contact with pathogens in the environment—such as sanitation interventions.

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INTEGRATING WATER SANITATION AND HYGIENE PRACTICES AND NEGLECTED TROPICAL DISEASE INTERVENTIONS: EXPERIENCE FROM SOUTHERN TANZANIA

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Tanzania is endemic for NTDs particularly the 5 PCT NTD,Trachoma being one of them. Efforts for control and elimination of The NTDs are underway since 1990s,they include MDA, Health education and Promotion and Morbidity Control. Following mapping, Trachoma was found to be endemic in 54 districts in year 2004 -2006 surveys and Mass treatment started in a phased approach. As per WHO a comprehensive SAFE strategy is paramount for Trachoma elimination but there has been some limitations due to several factors. Moreover a clear assessment of Integration of Water Sanitation and Hygiene (WASH) practices has not been well assessed. In 2014, The programme designed a project to assess and operationalize Integration between WASH and NTDs Interventions in two districts of Tunduru and Namtumbo of Ruvuma region, Southern Tanzania. Baseline sanitation data was collected through house to house visits using a questionnaires as well as from sanitation registers. Trachoma prevalence for Tunduru was from the surveys and most updated data shows a TF 7.20%, TT 1.2% while Namtumbo TF of 2.20%, TT 1.10% in 2014. Local artisan Training was conducted to all communities of the two councils and 20 artisans were trained. At each level a combination of two people was made in each visit an NTD personnel and WASH personnel. Assessments done after 1 year indicates both ; Increased number of Households with improved latrines from 603 to 2015 households out of 8063 total of households in Namtumbo and Tunduru. Moreover, households with fixed hand wash facilities increased from 57 to 1698 household in the period of March November 2015.For Trachoma Prevalence Namtumbo district has passed the threshold of <5% TF prevalence and thus do not require MDA for zithromax. These results signifies good cooperation/collaboration between government officials, partners and councils at all levels and the 2 districts can meet Trachoma elimination targets if integration of sanitation and hygiene with NTD interventions is strengthen.

1797

CHOLERA AND ENVIRONMENTAL DYNAMICS IN AN ECUADOREAN ESTUARINE SYSTEM

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The emergence of waterborne diseases such as cholera, whose causative agent is pathogenic strains of *Vibrio,* is strongly linked to the local environmental and ecological context. Machala is a port city of 250,000 people in El Oro province, on the southern coast of Ecuador, near the Peruvian border. The 1991-2004 cholera pandemic emerged in Peru and spread north into El Oro, making it a key sentinel site for understanding dynamics in the ongoing 7th pandemic. In Machala, many peoples' livelihoods depend on the estuarine system, from fishing for subsistence and trade, to domestic water use, making the coupled human-estuarine system an important component of public health management. We sampled five estuarine locations twice weekly over a 10-month span, within a gradient of human use, and over a geographic range from inland to ocean, to measure water-specific environmental variables such

as pH, temperature, salinity, conductance, and algal concentration, and conducted PCR testing for *Vibrio spp.*, including pathogenic strains, across 5 months. Our sites exhibited considerable seasonal and spatial heterogeneity in environmental variables, with clear peaks during specific months. We found evidence of an environmental reservoir for *Vibrio spp.*, including pandemic strains O1 and O139, but did not confirm ongoing toxigenic presence. We found that the timing of positive PCR results was coupled to the environment. This study was conducted in a moderately normal climate year, providing a preliminary framework for monitoring coupled *Vibrio* – estuarine dynamics for potential emergence of cholera outbreaks in the region.

1798

GLOBAL TRACHOMA MAPPING PROJECT: SANITATION COVERAGE THRESHOLD LEVELS AND PROTECTION AGAINST TRACHOMA

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Improved sanitation is thought to reduce trachoma by reducing the number of fly breeding sites. No study has attempted to characterize the thresholds of sanitation for trachoma; particularly, if there is a lower sanitation coverage threshold required to reduce trachoma, and also if there is some upper threshold at which sanitation might reduce trachoma even for those who don't use latrines through herd protection. We used data from the Global Trachoma Mapping Project (GTMP), collected between December 2012 and January 2016. To date, we have included data from 325,315 participants from 5 countries; additional data will soon be available from 17 countries in sub-Saharan Africa, Oceana, Asia, and Central and South America. Data cover all endemic districts in these countries using cluster sampling. Participants were surveyed about WASH access and practices and both eyes were examined for trachomatous inflammation - follicular (TF), trachomatous inflammation - intense (TI), and trachomatous trichiasis. Our outcome was combined TF/TI in either eye (binary), and the primary exposures of interest were household-level access to improved sanitation, and community-level prevalence of improved sanitation. Community-level access was defined as the prevalence of sanitation aggregated at the cluster-level. We employed multivariable mixed-effects modified Poisson regression models to jointly assess the relationship between household and community toilet coverage on TF/TI prevalence. We graphically show and present the relationship between the trachoma outcomes and individual and community sanitation coverage. The trachoma prevalence from adjusted models was generally lowest among latrine owners who were also are in the top latrine coverage quartile (prevalence = 1.5%; 95% CI: %1.3-%1.7). Our study provides insights into the sanitation thresholds required to reduce trachoma and our findings will have considerable public health and policy implications for achievement of elimination of blinding trachoma by 2020.

1799

USE OF MULTI-PARALLEL QUANTITATIVE REAL-TIME PCR FOR GASTROINTESTINAL PARASITES IN RURAL MOZAMBIQUE: CORRELATION OF INFECTION INTENSITY TO WATER ACCESS, SANITATION AND HYGIENE (WASH)

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Sub-Sahara Africa has the highest rates of intestinal parasites worldwide. More than 30% of children in these regions have a parasitic infection. These infections have the potential to cause morbidity and are related to environmental conditions. Our objectives were to deploy the first time use of multi-parallel quantitative real-time PCR (qPCR) to describe epidemiology of common soil-transmitted helminths and protozoa, and association with living conditions. This study involved 250 children (ages 1 to 10 years old, Geomean = 4.98 years old) residing in rural areas in Manhiça, Mozambique. Participants presented for routine care, stool samples obtained, and extensive questionnaire on water access, sanitation and hygiene (WASH), clinical and laboratory data were obtained for all enrollees. DNA extraction of stool and qPCR was performed at the Centro de Investigação em Saúde de Manhiça (CISM). qPCR detected 65% of children with 1 or more parasites, these include *Giardia lamblia* 61%, *Ascaris lumbricoides* 10.2%, *Strongyloides stercoralis* 8.6%, *Cryptosporidium* 4%, *Necator americanus* 2.8%, *Ancylostoma duodenale* and *Entamoeba histolytica* were not detected, *Trichuris trichiura* results are pending. Concentration of *Ascaris* DNA was converted to eggs per gram (EPG) via previous correlation studies. Greater than 60% had heavy *Ascaris* EPG burdens. Heavy *Ascaris* EPG burden correlated with increased *Giardia* DNA burden in co-infected children (p = 0.0177). Preliminary data points to a higher prevalence of helminth and protozoal infections than previous known. Ongoing analysis will correlate WASH data to qPCR prevalence and co-infections, associating the lack of sanitation to higher rates and intensities of infections. These studies improve our understanding of the interaction between sanitation and parasitic infections, and build capacity for ongoing public-health initiatives in endemic regions.

1800

EFFECT OF A COMBINED HARDWARE AND BEHAVIOR CHANGE INTERVENTION ON HANDWASHING BEHAVIORS IN PRIMARY SCHOOL CHILDREN: THE POVU POA SCHOOL PILOT STUDY

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Kenyan schools often lack bar soap for handwashing and experience water scarcity. Soapy water can deter soap loss and is inexpensive. A behavior change intervention based on disgust and promoting social norm change increased handwashing in a community setting, but effectiveness in schools has not been assessed. In Kenyan public schools, we tested Povu Poa: a handwashing station with a dispenser that produces foam from soapy water along with a behavior change intervention for schoolchildren with disgust-based triggers and social norm change components. In a stepped-wedge cluster-randomized trial, we assessed effects of the intervention on 1) availability of handwashing materials, and 2) handwashing behavior after toilet use among schoolchildren. We randomly selected 30 schools in Kisumu and divided them into 3 groups of 10 schools. After baseline data collection, we delivered the intervention sequentially (Group 1: 3-5 weeks post-baseline; Group 2: 6-8 weeks; Group 3: 19-24 weeks). We observed outcomes at baseline and at Round 1: 3-5 weeks after baseline, Round 2: 9-12 weeks, and Round 3: 20-28 weeks. We compared outcomes at schools prior to intervention (Comparison Group) to outcomes at schools after intervention (Intervention Group). Water and soap / soapy water or foam were available at <1% of handwashing places in the Comparison Group, and at 50% of handwashing stations in the Intervention Group. In the Comparison Group, we observed handwashing with water after 13% of toilet use events; we did not observe any handwashing with soap. In the Intervention Group, we observed handwashing with water after 36% of toilet use events (RR = 5.14, 95% CI = 2.55, 10.34) and handwashing with foaming soap after 32% of the events (RR incalculable because there was no handwashing with soap in the Comparison Group). The Povu-Poa intervention increased handwashing in schoolchildren, although a sizable proportion of toilet use events were not followed by handwashing with soap. Investigation of barriers to both maintenance of the soap foamer and adherence to handwashing with foaming soapy water after toilet use would inform improvements in intervention design.

1801

EVALUATING THE IMPACT OF SCHOOL WATER, SANITATION AND HYGIENE IMPROVEMENTS USING THE PRESENCE OF SERUM ANTIBODIES FOR ENTERIC AND NEGLECTED TROPICAL DISEASES AMONG SCHOOL CHILDREN IN MALI

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The purpose of this study was to evaluate the biologic impact of school WASH improvements on enteric and NTD incidence among pupils in Mali. We piloted the use of dried blood spots (DBS) from school-aged children (SAC) to detect blood serum antibodies for 32 analytes of enteric and NTDs using a Luminex multiplexing assay. This method has yet to be employed among SAC, and has had limited employment in evaluation of WASH trials. We randomly selected 21 beneficiary schools participating in an evaluation of a comprehensive school-based WASH intervention in Mali, and their 21 matched control schools. In each school, 20 pupils were randomly selected and interviewed about their household WASH access, school absence, and recent illness. Capillary whole blood in the form of DBS was collected from each student. DBS were analyzed for blood antibodies for E. histolytica, Giardia, *Cryptosporidium*, *P. falciparum*, *P. vivax*, dengue, Chikungunya virus, *E. coli*, cholera, Salmonella, norovirus, *Campylobacter*, filariasis, Strongyloides, trachoma, cysticercosis, and schistosomiasis using a Luminex multiplexing assay. Data were analyzed using generalized linear latent and mixed models (GLLAMM). Factor analysis identified three distinct latent variables representing vector-transmitted disease, food- and water-transmitted disease, and person-to-person transmitted disease. The GLLAMM modeling framework consisted of a measurement model of these three latent variables, clustered at the school level and controlling for pupil age, and a structural model of the regression of intervention and household WASH access on the latent variables. Food/water transmitted disease and person-to-person transmitted disease was lower among pupils attending intervention schools. Vector transmitted disease was higher among pupils attending intervention schools. Results from this pilot support findings from the impact evaluation of the larger trial, which found reduced incidence of diarrhea among pupils attending beneficiary schools. Analysis of DBS is promising method to objectively evaluate WASH impacts in low-resource field settings.

1802

PREVALENCE OF ANTIBIOTIC-RESISTANT BACTERIA AND THEIR RESISTANCE GENES IN SURFACE WATERS IN A RURAL COMMUNITY OF BRAZIL

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Brazil uses less antibiotics for humans than the USA, but approximately the same amount in agriculture. We therefore studied the antibiotic resistance profile of a river system in a rural community of Bahia, Brazil. In this population of ~500, all homes have a flush toilet, but 50% of these flush to the river instead of a septic tank. Agriculture is the principle occupation, and cattle, pigs and chickens are kept within and around the community. In contrast to the USA, quinolones are not added to feed, except for treatments. River water was collected from 10 points where the population commonly has water contact as well as 2 samples of the local piped water. Monthly records of water quality and climate were made for all sites including physical characteristics, coliform count and qPCR for fecal microbial source tracking (MST). Bacterial susceptibility to ciprofloxacin, cefotaxime and meropenem was tested by disk diffusion. DNA was extracted from filter retentate of 500 ml collected from each site to identify known quinolone, cephalosporin and carbapenam resistance genes by PCR. Coliforms and *E. coli* were found at high concentrations at all sites. By contrast, MST indicated that the highest concentration of human fecal waste was downstream from the population center, and diminished 500 m downstream of the last home. In July of 2015, all sampled points showed at least one bacterial colony resistant to 1 of the 3 antibiotics. Four Enterobacteriaceae with resistance to at least 1 antibiotic were isolated, including one from the drinking water supply. Citrobacter freundii which proved to be positive for an extended beta-lactamase (ESBL) was found in one of the points. PCR assays for 4 common quinolone resistance genes, a cephalosporinase gene and 6 carbapenamase genes were negative at all sites. These results indicate that MST may provide a more reliable estimate of human fecal contamination; that surface waters in this rural community do not meet national standards for human contact and the drinking water was doubtful for consumption. Yet the limited use of quinolones in veterinary practice may explain the absence of resistance genes in these waters.

1803

FECAL FINGERPRINTS: THE LANDSCAPE OF ENTERIC PATHOGEN CONTAMINATION IN LOW-INCOME, URBAN NEIGHBORHOODS OF KISUMU, KENYA

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Children in developing countries are infected with a variety of enteropathogens in the first years of life, suggesting they experience complex environmental exposure risks. Little is known about how different fecal transmission pathways contribute to enteric infection patterns. During an exposure assessment, the Social Microbes study characterized the spatial distribution and correlative relationships for twenty-two types of enteropathogens in three low-income, urban neighborhoods of Kisumu, Kenya. Landscape features, condition of latrines, evidence of open defecation, zoonotic vectors, and number of children and their behaviors were recorded at sixty randomly-selected sites in each neighborhood (N=180). Soil and surface water from each site was analyzed using enterococci indicator assays. A microfluoridic qPCR tool was used to detect twenty-two enteric viruses, bacteria, or parasites in nucleic acid extracted from 0.5 grams of soil and 10 mls of surface water. Enterococci were detected in 100% of surface water samples (N=26) and 73% of soil samples (N=114) in two neighborhoods. Enteropathogens were detected in 92% of water samples (5.1 enteropathogens/sample) and 84% of soil samples (1.2 enteropathogens/sample). The five most common pathogens in water were *Cryptosporidium* spp. (89%), Enteroaggregative *E. coli* (EAEC) (77%), Enterotoxigenic *E. coli* (ETEC) ST/LT (54%), human adenovirus (50%), and *Giardia lamblia* (42%). Fourteen other enteropathogens were detected at lower frequencies. The five most common pathogens in soil were *Cryptosporidium* spp. (70%), *G. lamblia* (16%), EAEC (8%), human adenovirus (7%), and ETEC-LT (6%). Twelve other enteropathogens were detected at lower frequencies. Multidimensional correlation between enteropathogens was observed in water, but not soil. The variety and prevalence of specific enteropathogens detected in environmental fomites in Kisumu provides powerful clues for explaining the etiological complexity of pediatric enteric infection in developing countries, and highlights the need for improved exposure assessment methods for identifying fecal transmission pathways.

1804

MULTI-PARALLEL QUANTITATIVE REAL-TIME PCR FOR GASTROINTESTINAL PARASITES AND INFECTION BURDEN IN DISTINCT COLOMBIAN COMMUNITIES

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Gastrointestinal (GI) parasites are globally widespread infectious agents disproportionately affecting children in resource-deprived areas with associated morbidity that is poorly understood. Environmental surroundings influence exposure to these parasites as does the differences of water access, sanitation, and hygiene (WASH) between different community settings (urban, peri-urban, rural). Stool samples from 194 children in a urban slum (n = 72, mean age = 2.5 yrs), peri-urban (n = 50, mean age = 6 yrs), and rural (n = 72, mean age = 2 yrs) areas were analyzed using multi-parallel quantitative real-time PCR (qPCR) for *Ascaris lumbricoides, Ancylostoma duodenale, Necator americanus, Trichuris trichiura, Strongyloides stercoralis, Cryptosporidium, Entamoeba histolytica*, and *Giardia lamblia*. Prevalence was 62.5% *Giardia*, 23.6% *Cryptosporidium*, 19.4% *Ascaris*, and 5.5% *Trichuris* in urban slum; 26.4% *Giardia* and 2.7% *Ascaris* in peri-urban area; and 68% *Giardia*, 20% *Entamoeba*, 50% *Ascaris*, 46% *Trichuris*, and 2% *Strongyloides* in rural area. Children infected with polyparasitism (2 or more parasites) correlated to living in rural areas compared to urban and peri-urban (59%, 19%, 0%, p = 0.001). Prevalence was lowest in peri-urban area, likely due to less exposures in older age group. Higher *Giardia* DNA burden correlated to living in urban slums (p = 0.008); potentially due to crowding and sharing contaminated water. Helminth burden was correlated to eggs per gram (EPG) with higher *Trichuris* burden found in rural areas compared to urban slum (9953 versus 325 EPG, p = 0.0023). Over 40% of helminth infections in rural area are classified as heavy burdens by WHO classes of intensity. Our data is useful for morbidity studies and public health interventions in highlighting need for improvements in WASH infrastructure. Ongoing work will contribute *Giardia* and *Cryptosporidium* spiking studies correlating trophozoite/cyst to burden. Future work will correlate parasite DNA to clinical outcomes and explore associations with childhood morbidity.

1805

ANTIBIOTIC STEWARDSHIP AND SANITATION: A MISSING PARTNERSHIP

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Antimicrobial resistance is a major global public health problem by limiting treatment of patients infected with multi-resistant bacteria. We evaluated the presence of resistance genes for quinolones (qnrA, qnrS, aac(6'), oqxA), cephalosporins (blaCTX-M) and carbapenems (blaOXA-48, blaVIM-2, blaNDM-1, blaKPC, and blaSPM) from a lake (DC) in a major urban center in Brazil (Salvador, Bahia) compared with a river system in a rural community in the state of Bahia, Brazil, a Lake (SL) in Cleveland, Ohio and the Cleveland sewer system. Water was sampled from DC in 2013 and 2015. All other sites were sampled in 2015. The 500 ml samples were filtered through a 0.22 µm pore nitrocellulose filter and DNA extracted with phenol-chloroform. Standard PCR assays were used to identify antibiotic resistance genes. Bacterial source tracking in DC showed high human fecal contamination similar to Cleveland sewage. For the DC in 2013, 2/15 samples were positive for OXA-48 and 7/10 in 2015. Of the 7 sites positive for OXA-48, 3 were also KPC positive. VIM-2 was identified at 2 sites. Quinolone resistance genes were found at multiple sites in 2013 for DC, but this analysis is pending for 2015. The sample taken from the sewer system of Cleveland was positive for only VIM-2. Few or no resistance genes were identified in river samples from the rural community in Brazil and the SL in the USA. From the latter, only 1 bacterial isolate was resistant to any antibiotics tested in contrast to all other locations where there were numerous resistant isolates. The earliest report of the OXA-48 gene family in Enterobacteriaciae in Brazil was from 3000 km south of Salvador in a hospital in Porto Alegre in 2013. This gene had, however, clearly entered the country earlier and was already widely disseminated in the environment in 2013. The frequency and number of antibiotic resistance genes in DC is alarming but not unusual for urban surface water that is used by the community for recreation and fishing. The presence of poor sanitation and feces in urban surface water is likely an important factor contributing to the spread of these genes to many bacterial species and back to humans.

1806

WHO INFLUENCES YOU? THE ROLE OF WOMEN IN INFORMATION DIFFUSION OF SANITATION AND WATER PRACTICES IN COASTAL ECUADOR

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Despite dramatic reductions in childhood mortality in the past decade, diarrhea remains a major cause of preventable childhood deaths worldwide. Aside from vaccination, well known measures to prevent diarrheal infection include good water, sanitation, and hygiene practices. These behavioral practices, however, are influenced by a multitude of factors, including community-level social cohesion. Women, in particular, experience a continual tradeoff in daily tasks, including water-associated behaviors and child care, and likely play a role in influencing information diffusion in societies with high social cohesion. Previous studies conducted on coastal Ecuadorian populations have shown that a greater density of social ties between individuals in remote communities may lead to the spread of sanitation and water practices, both individual and collective, that help reduce the transmission of diarrheal disease. The role of women and the effect across time were not examined. We aim to examine the influential role women play on information diffusion, as defined by adopting improved sanitation and water practices, and diarrheal disease reduction in coastal Ecuador over the course of ten years. Using longitudinal social network data collected from villages in northern coastal Ecuador at multiple intervals from 2003 to 2013, we first defined communities with high and low social cohesion by measures of network density and clustering. We then measured node centrality, including average degree, closeness, and betweenness, by gender in networks of high and low social cohesion to examine the presence of influential nodes. We also assessed the presence of strong and weak ties. We conducted Markov-chain Monte Carlo models to determine the influence of women on the effect of high social cohesion on changes in sanitation and water practices and diarrheal disease over time. Qualitative data was used to describe difference in the role of women in communities of low and high social cohesion. By understanding who the influential persons are in social networks, we can better understand how to leverage social learning to reduce diarrheal disease transmission.

1807

CLINICAL PREDICTION RULE OPERATED BY MOBILE PHONES FOR EARLY DETECTION AND REFERRAL OF CUTANEOUS LEISHMANIASIS IN RURAL AREAS OF COLOMBIA

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Detection and diagnosis of cutaneous leishmaniasis (CL) in rural populations is a public health challenge. A Clinical Prediction Rule (CPR) previously validated in Tumaco could provide presumptive diagnosis of CL using 6 readily obtained variables. We sought to adapt the CPR as mobile phone app to facilitate case detection in rural areas and evaluate users performance and acceptability. 6 community volunteers and 3 health technicians were trained in the use of "Leishmaniasis App". During Feb 2015 to Mar 2016 patients with skin lesions were evaluated with the CPR and received parasitological tests. Number of confirmed cases and time from symptoms onset to diagnosis during were compared with data reported by the national surveillance system during 2012-2014. Agreement between community volunteers and health technicians with an experienced physician was estimated. Semi-structured interviews and focus group were used to evaluate users' acceptance and usability of the app. A total of 115 patients were evaluated, 83.5% had parasitological confirmation and 16.5% other dermatologic conditions. Confirmed cases increased 27% during the study period compared to years 2012-2014 (213 vs 167) and average time from symptoms onset to confirmed diagnosis decreased 53.8%, from 30.4 to 11 days. Overall agreement between the experienced physician and community volunteers was 93.8% (Kappa:0.68) and 96.8% (Kappa:0.72) for health technicians. Variables referred by patients (i.e. risk activities, vector contact and trauma) had ≥ 87.5% agreement. Presence of clustered lesions had agreement ranging 56.3% to 100%. Ninety percent of users fully agreed with usefulness of the app and 72% considered use of mobile phones easy and relevant. Main perceived barriers were cultural differences of indigenous communities and armed groups. Mobile phone use was facilitated by familiarity with technology and relevance of having an appropriate tool for CL detection. The use of a mobile app adapting a validated CPR by community volunteers and health technicians evidences the utility and acceptability of an m-health tool for presumptive diagnosis of CL in rural communities.

1808

THE EFFECT OF TEXT MESSAGE REMINDERS TO HEALTH WORKERS ON QUALITY OF CARE FOR MALARIA, PNEUMONIA, AND DIARRHEA IN MALAWI: A RANDOMIZED CONTROLLED TRIAL

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Mobile (mHealth) technologies hold promise as innovative ways to improve health worker (HW) performance in low-resource settings. We conducted a cluster-randomized controlled trial to evaluate the effect of text message reminders to HWs in outpatient health facilities (HFs) on the quality of care for malaria, pneumonia, and diarrhea in Malawi. After a baseline HF survey in January 2015 with patient interviews, HF assessments, and HW interviews, 105 HFs were randomized, stratified by baseline quality of care, to three arms: 1) text messages to HWs on malaria case management; 2) text messages to HWs on malaria, pneumonia, and diarrhea (latter two for children <5 years); and 3) control arm (no messages). Messages were sent twice a day for six months, followed by an end-line HF survey in November 2015. Difference-of-differences logistic regression analyses, accounting for clustering at facility level, were performed. We interviewed 2,360 patients at baseline and 2,536 at end-line. The proportion of patients with suspected uncomplicated malaria managed correctly increased from 40.3% to 52.8% in the control arm, from 41.4% to 55.6% in arm 1 (effect size 1.7%-points, p=0.84), and from 32.9% to 53.5% in arm 2 (effect size 8.1%-points, p=0.34). Prescription of first-line antibiotics to children <5 years with clinically-defined pneumonia increased from 69.1% to 70.6% in the control arm, from 68.9% to 71.3% in arm 1 (effect size 0.9%-points, p=0.95), and from 69.6% to 76.5% in arm 2 (effect size 5.4%-points, p=0.68). Prescription of oral rehydration solution to children with diarrhea declined slightly in all arms from baseline to end-line. Per-protocol analyses limited to patients seen by HWs in arms 1 and 2 who reported receiving messages (39.5% and 45.5%, respectively) yielded similar results. We found no significant improvements in malaria, pneumonia, or diarrhea treatment practices after six months of twice-daily text message reminders to HWs, illustrating the importance of rigorously testing new interventions before adoption and understanding why interventions work well in some settings, but poorly in others.

1809

MULTIMEDIA TOOL FOR OBTAINING INFORMED CONSENT IN THE GAMBIA: A MIXED METHOD STUDY

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Communicating crucial research information to low literacy research participants in Africa is highly challenging in the context of several factors which make the participants vulnerable to poor comprehension of consent information. We previously developed and validated a multimedia consent tool and a digitized audio comprehension questionnaire. This study was undertaken to evaluate the effectiveness of the multimedia consent tool amongst adults participating in a clinical trial in The Gambia. Adults eligible for inclusion in a malaria treatment trial (n = 311) were randomized to receive information needed for informed consent using either a multimedia tool (intervention arm) or a standard procedure (control arm). A computerized, audio questionnaire was used to assess participants' comprehension of informed consent. This was done immediately after consent had been obtained (at day 0) and at subsequent follow-up visits (days 7, 14, 21 and 28). The acceptability and ease of use of the multimedia tool were assessed in focus groups. On day 0, the median comprehension score in the intervention arm was 64% compared with 40% in the control arm (P = 0.042). The difference remained significant at all follow-up visits. Poorer comprehension was independently associated with female sex (odds ratio, OR: 0.29; 95% confidence interval, CI: 0.12-0.70) and residing in Jahaly rather than Basse province (OR: 0.33; 95% CI: 0.13-0.82). There was no significant independent association with educational level. The risk that a participant's comprehension score would drop to half of the initial value was lower in the intervention arm (hazard ratio 0.22, 95% CI: 0.16-0.31). Overall, 70% (42/60) of focus group participants from the intervention arm found the multimedia tool clear and easy to understand. In conclusion, a multimedia tool significantly improved comprehension and retention of consent information by research participants with low levels of literacy in The Gambia. Further evaluation of the tool is warrranted in similar settings.

1810

FIRST ORAL CHOLERA VACCINATION CAMPAIGN IN IRAQ DURING AN OUTBREAK AND HUMANITARIAN CRISIS: FINDINGS FROM THE COVERAGE SURVEY, 2015

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As part of the 2015 cholera outbreak response in Iraq, the Iraqi Ministry of Health (MOH) targeted approximately 255,000 persons aged ≥1 year living in selected refugee camps, internally displaced persons (IDP) camps, and collective centers with two doses of oral cholera vaccine (OCV) during November-December 2015. This was the first use of the OCV in Iraq and the largest global OCV stockpile deployment to date. We conducted a multi-stage cluster survey to obtain OCV coverage estimates in 10 governorates that were targeted during the 2015 campaign. Within each governorate we proportionally allocated our sample based on the estimated population size of each refugee/IDP camp or collective center; approximately 120 households were systematically sampled in each governorate. In each selected household, all persons aged ≥1 year were interviewed. In total, 1,226 household and 5,007 individual interviews were conducted. Overall, two-dose OCV coverage in the targeted camps was 87% (95% CI: 85%, 89%). Coverage was similar across age groups; 85% (95% CI: 81%, 88%) among children 1-4 years old, 89% (95% CI: 85%, 92%) among children 5-14 years old, and 87% (95% CI: 84%, 90%) among persons aged ≥15 years. Two-dose OCV coverage was higher in the three Northern governorates at 91% (95% CI: 89%, 93%) (range: 89% (Dahuk) to 93% (Erbil and Sulaymaniya)) compared with the seven South and Central (S/C) governorates at 80% (95% CI: 77%, 82%), where greater variation between governorates was noted (range: 21% (Babil) to 98% (Anbar)). Lower two-dose coverage in S/C governorates were likely due to civil strife, heavy rains, and challenges in program management. One-dose only coverage was higher (10%; 95% CI: 8%, 12%) among the S/C governorates compared to the Northern governorates (6%; 95% CI: 4%, 9%). The most common reasons for not receiving OCV was being absent during the campaign or teams not visiting their homes. No serious adverse events following immunization were reported. The Iraq experience demonstrates that OCV campaigns can be successfully implemented as part of a comprehensive response to cholera outbreaks among high-risk populations in conflict settings.

1811

TIMELINESS OF VACCINATION IN AN URBAN SLUM IN NAIROBI, KENYA

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Although routine infant immunization programs achieve high coverage in the first year of life in many low-resource settings, delays in vaccine receipt can leave very young children at risk for preventable diseases, precisely when they are at greatest risk for severe infection. Children living in urban slums are vulnerable to many diseases because of precarious living conditions and high population density. We assessed timeliness of vaccination among a cohort of fully vaccinated children within a population-based surveillance platform in Kibera, an urban slum in Nairobi, Kenya. Surveillance participants (~25,000) were visited at home biweekly and received free care for acute illness at a designated clinic. At each visit parents were queried about vaccines children had received since the prior visit; reported doses were verified using the child's immunization card. We identified all children <5 years old with 3 card-confirmed doses of pentavalent (diphtheria-tetanus-pertussis-Haemophilus influenzae type b-hepatitis B) vaccine, which is given at 6, 10, and 14 weeks in Kenya. We used inverse Kaplan Meier curves and Cox proportional hazards models to identify factors associated with timely receipt of the 3rd dose. From December 2009 to December 2014, 1,874 children received 3 pentavalent doses. The mean and median age at receipt of the 3rd dose was 17 and 15 weeks. The proportion with 3 doses by 4, 6, 12, and 24 months was 76.1%, 95.3%, 98.2% and 99.6% respectively. Timeliness of vaccination was not significantly associated with sex, birth in rainy season, or household size. Residence in a geographic zone close to the clinic was associated with delayed vaccination (HR=0.87 95%CI: 0.77-0.99) and birth in December was associated with timely vaccination (HR=1.24; 95%CI: 1.04-1.48). We found receipt of pentavalent vaccine to be quite timely among children who eventually received 3 doses. Although nearly a quarter were missing at least one dose at the age of 4 months, by 6 months >95% had received all 3 doses. The relevance of identified factors associated with timeliness is unclear, particularly given the small numbers of children with substantial delay.

1812

COST-EFFECTIVENESS OF DENGUE VACCINATION IN FIVE LATIN AMERICAN COUNTRIES

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In 2015, the first dengue vaccine was licensed in several Latin American countries, providing a promising tool against an expanding disease. However, decisions about the vaccine's use depend on quantifying its health benefits, costs, and cost-effectiveness. To inform policy discussions in Latin America, we used a transmission model calibrated with data from Phase III efficacy trials. Costs of vaccine administration, procurement, and dengue treatment were based on publications and reports. Each vaccine dose was projected to cost $2 for vaccine delivery plus $20 for vaccine procurement. Our base case assumed that a 3-dose vaccination program would be offered to all 9 year-old children each year, plus a 4-cohort initial catch up (10-13 year-olds), phased over 3 years and achieving 80% coverage. Our base case expressed costs in 2013 US dollars from a health system perspective, conducted 100 simulations with a 30-year horizon to account for variability in dengue transmission and uncertainty on vaccine efficacy, measured health impacts in disability-adjusted life years (DALYs), and assessed cost effectiveness as $/DALY averted. Our base case results found that vaccination would save from $0.19 (Honduras) to $1.91 (Puerto Rico) in annual per capita dengue treatment costs and would reduce dengue-related DALYs by 27.4% (Mexico) to 32.2% (Honduras). Cost-effectiveness ratios, expressed as multiples of each country's per capita gross domestic product (GDP), were: Brazil (0.74), Colombia (0.27), Honduras (3.58), Mexico (0.18), and Puerto Rico (-0.15). In the base case, the vaccine is cost saving in Puerto Rico. Using WHO benchmarks of 1 and 3 times per capita GDP, the vaccine is highly cost effective in Brazil, Columbia and Mexico (under the most stringent benchmark), but not cost effective in Honduras from a health system perspective. Cost-effectiveness results were similar for other programs (0 to 8 catch up cohorts) and coverage rates (50% to 80%). The consideration of a societal perspective, increasing dengue incidence, dengue's adverse impacts on tourism, and rising real incomes and health care costs further strengthen the case for vaccination.

1813

SNAKEBITE: STRATEGIES TO REVERSE THE PUBLIC HEALTH NEGLECT OF TROPICAL SNAKEBITE VICTIMS

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Globally, snakebite kills one fifth the number of people that die from malaria. In India, half the number of people dying from HIV are killed by snakebite every year. In Africa, snakebite causes nearly twice the number of deaths, every year, than the recent Ebola epidemic - and imposes a disease burden (319,874 DALYs; 16 countries) equal or exceeding that of regional NTDs such as Buruli ulcer, Echinococcosis, Leishmaniasis, Trachoma and Trypanosomiasis. Surviving snakebite victims suffer substantial psychological morbidity that is typically unrecognised and untreated. The support and investment provided by International Health Agencies and tropical governments to greatly reduce the disease burden of malaria, HIV, Ebola and the NTDs is typically denied to tropical, and particularly to sub-Saharan African, snakebite victims - despite the high mortality rate and the physical, psychological and socio-economic burden of tropical snakebite. In an effort to reverse this public health neglect of tropical snakebite victims, the authors organised (September 2015) a Wellcome Trust-funded workshop to identify key interventions (i) reduce snakebite incidence, (ii) improve access to hospital care, (iii) improve clinical management of hospitalised snakebite victims and (iv) improve post-hospital management of snakebite victims. We will report that progress since then includes (i) the announcement by the World Health Organisation of an 'African antivenom prequalification' program designed to prevent the distribution in Africa of ineffective antivenoms, (ii) that the NGO, Health Action International, has assumed the secretariat and advocacy roles for the Global Snakebite Initiative, (iii) that, with the advocacy support of over 13 tropical MoHs and NGOs (eg, MSF, HAI, DNDi), the Global Snakebite Initiative acquired a side briefing at the World Health Assembly in May, 2016, (iv) that a motorcycle ambulance/smart phone app-coordinated Snakebite Emergency Response System will be trialled in Kenya as an affordable, rapid means of delivering rural snakebite victims to effective treatment.

1814

A NOVEL FAMILY OF KUNITZ-TYPE INHIBITORS FROM *FASCIOLA HEPATICA* - POTENT INHIBITION OF VIRULENCE-ASSOCIATED CYSTEINE PROTEASES

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Fasciola hepatica is a zoonotic food-borne helminth parasite of global veterinary and medical importance. The parasite expresses a family of seven Kunitz-type (KT) protease inhibitors that are highly regulated during the parasites migration and development in the mammalian host. Phylogenetic analysis demonstrates they separate into five subgroups (FhKT1 – 5), although transcriptomic data shows that the FhKT1 group has the highest expression over the course of infection and is upregulated in the infectious newly excysted juveniles (NEJs) and in adults. To date, KT inhibitors are ubiquitously expressed in eukaryotics and are classical described as inhibitors of serine proteases. Unexpectedly, we discovered that the FhKT1 inhibitors do not inhibit serine proteases but exhibit potent inhibition towards cysteine proteases. Recombinant FhKT1 is a potent inhibitor of the major secreted virulence-associated cathepsin L cysteine proteases of *F. hepatica*, FhCL1, FhCL2 and FhCL3, and of human cathepsins L and K (K_i = 0.2474 nM – 24.607 nM). FhKT1 also prevented the auto-catalytic activation of FhCLs and formed stable complexes with the mature enzymes. Pull-down experiments showed that rFhKT1 interacts specifically with native secreted adult FhCL1, FhCL2 and FhCL5. Substitution of an unusual P1 Leu^{15} within the exposed reactive loop of FhKT1 for the more commonly found Arg^{15} (FhKT1Leu^{15}/Arg^{15}) had modest adverse affects on cysteine protease inhibition but conferred potent activity against the serine protease trypsin (K_i = 2.28 nM). Computational docking and sequence analysis provided molecular explanations for the exclusive binding of FhKT1 to cysteine proteases, suggested a pivotal role for the P1 Leu^{15} in anchoring the inhibitor into the S2 active site pocket, and helped explain the selectivity towards cathepsin L-like proteases. FhKT1 represents a novel evolutionary adaptation of KT protease inhibitors by *F. hepatica*, with its prime purpose likely in the regulation of the major parasite-secreted proteases and/or host proteases during infection, making this a novel vaccine and drug target.

1815

DRAFT GENOMES OF FOUR SPECIES OF THE LUNG FLUKE *PARAGONIMUS*

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Paragonimus spp, the lung fluke, is among the most injurious of the food-borne helminths, infecting ~20 million people worldwide, with an estimated 293 million people at risk for infection. Paragonimiasis is acquired by consuming raw or undercooked crustaceans containing *Paragonimus* metacercariae, and primarily affects the lungs, but often causes lesions elsewhere in the body, including the brain. The disease is a major public health concern in parts of Southeast Asia, West Africa, South and Central America, and Northeast India, where it is frequently mistaken for tuberculosis due to its similar respiratory symptoms. To substantially improve our understanding of pathogens across this genus at the molecular level, we have assembled, annotated and compared draft genomes of three *Paragonimus* species from Asia (*P. miyazaki*, *P. westermani*, *P. heterotremus*) and one from North America (*P. kellicotti*). The genomes range in size from 697 to 923 Mb, contain between 11,761 and 12,762 genes, and are estimated to be between 80% and 91% complete. Comparative orthologous protein family (OPF) analysis spanning 19 species (4 *Paragonimus* species, 3 other foodborne trematodes, 3 schistosomes, 4 other platyhelminths, 4 hosts and an outgroup) identified proteins and functions of phylogenetic interest, including 364 OPFs conserved across and specific to the four *Paragonimus* species, which were enriched for proteins responsible for transcription factor activity, iron homeostasis, and serine endopeptidase activity. Transcriptomic analysis identified gene sets with conserved expression across *Paragonimus* species, as well as genes overexpressed during host parasitic stages, including 179 *P. miyazaki* genes overexpressed in peritoneal and pleural cavities compared to liver and lung tissues, which were enriched for cysteine endopeptidase activity and microtubule processes. This study provides a foundation for future studies of *Paragonimus* and other food-borne trematode pathogens, and represents a major contribution to ongoing trematode genome sequencing efforts.

1816

COMPLEMENTATION OF CELLULAR PROLIFERATION DRIVEN BY GRANULIN BY LIVER FLUKE GRANULIN IN A CHOLANGIOCYTE LINE AFTER GENOME EDITING TO MUTATE HGRN

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The highest incidence of cholangiocarcinoma (CCA), bile duct cancer, has been reported in northeastern Thailand, a region where infection with the fish-borne liver fluke, *Opisthorchis viverrini* is endemic. Infection with *O. viverrini* is a Group 1 biological carcinogen that induces CCA. How opisthorchiasis causes CCA is not yet clear, but likely is a multi-factorial process. Among other factors, *O. viverrini* secretes a mitogen termed granulin (Ov-GRN-1) that stimulates proliferation of cholangiocytes, and we have postulated Ov-GRN-1 released from the parasites contributes to opisthorchiasis-induced CCA. Human orthologue, hGRN (human granulin) is a growth factor with multiple functions in inflammation, would repair, and tumorigenesis. To investigate these phenomena, we undertook complementation of native hGRN with Ov-GRN-1 in cultures of a cholangiocyte cell line named H69 where the encoding hGRN gene had been gene-edited out by CRISPR/Cas9. In real time growth assays (xCELLigence), mutant (hGRN knockout) cells exhibited reduced growth proliferation compared to wild-type H69 cells, a deficit that was relieved by addition of Ov-GRN-1. Thereafter, transcripts recovered from exosomes of H69 cells were evaluated; annexin, c-Myc, C-met were up-regulated. Moreover, perioxiredoxin, Prx I, an antioxidant involved with cellular homeostasis and which can promote tumorigenesis through activities driven via mTOR exhibited marked induction in the mutant H69 cells. In ongoing studies, intercellular functions of endogenous and exogenous granulin in cellular proliferation and/or tumorigenesis upstream of the mTOR pathway are under investigation.

1817

TISSUE SPECIFIC LOCALIZATION OF *NEORICKETTSIA* ENDOSYMBIONTS IN THE INTESTINAL TREMATODE *PLAGIORCHIS ELEGANS* AND THE LIVER FLUKE *FASCIOLA HEPATICA* SHOW SIMILAR DISTRIBUTION PATTERNS

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Neorickettsia are α Proteobacteria that can cause serious diseases in livestock animals and humans. These intracellular bacteria are transmitted by digenean trematodes but little is known about their relationship. *Neorickettsia risticii* has been isolated from infected horses, cultured in the laboratory, and described by transmission electron microscopy (TEM). However, ultrastructure and tissue localization of Neorickettsia in digeneans is largely unknown. We expressed a surface protein of Neorickettsia of *Plagiorchis elegans* (PeNsp-3) from experimentally infected hamsters, and raised antibodies to it for immunolocalization. TEM studies of *P. elegans* revealed pleomorphic bacteria with a median size of 600 x 400 nm and with characteristic double membranes. Bacteria secreted polymorphic vesicles into the host cell or cell syncytium. We used the PeNsp-3 antibody for comparative detection of Neorickettsia in adults of *P. elegans* (North Dakota) and *Fasciola hepatica* (Oregon). Neorickettsia from *P. elegans* and *F. hepatica* are closely related to each other and to N. risticii (Illinois). On the amino acid level PeNsp-3 is 98% identical to its ortholog of Neorickettsia from *F. hepatica*. Neorickettsia showed similar localization pattern in both trematode species. Endosymbionts were unevenly localized as single cells, or as small morula-like clusters in tegument, ovaries, vitelline glands, uterus, eggs, testis, seminal receptacle, intestine and oral and ventral sucker. Large numbers were present in the Mehlis' gland. Examination of hamster small intestine infected with *P. elegans* showed bacteria at the host-parasite interface of the oral and ventral sucker. We conclude that in *P. elegans* and *F. hepatica* large numbers of Neorickettsia in the Mehlis' gland and adjacent tissues involved in egg assembly participate in vertical transmission. Their presence in suckers and intestinal tissues may facilitate horizontal transmission to the host of the trematode. This first localization of Neorickettsia endsymbionts in adult trematodes of medical and veterinary importance provides important clues about their transmission modes.

1818

MATHEMATICAL MODELING OF THE TRANSMISSION DYNAMICS OF *OPISTHORCHIS VIVERRINI* IN LAO PDR

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The trematode liver fluke, *Opisthorchis viverrini*, which causes the chronic hepatobiliary disease, opisthorchiasis, is prevalent is southeast Asia. We develop a mathematical model of the transmission dynamics of *O. viverrini* through its life cycle in snails, fish, and humans; and a second model that includes potential transmission from reservoir hosts such as domestic cats and dogs. We calibrate these models to data collected from two communities in Khong Island in Southern Lao PDR. Analysis of the model assuming no reservoir hosts, shows that interventions such as behavioral changes in dietary habits (reducing transmission from fish to humans) and improved sanitation (reducing transmission from humans to snails) are most effective in reducing transmission potential and the mean burden of worms in humans. However, in the presence of reservoir hosts, snail control, if feasible, is the most effective intervention for reducing transmission potential, but behavioral changes in dietary habits remains the most effective intervention for reducing the worm burden in humans. Additionally, the model suggests that for the observed prevalence of infection in dogs and cats in Khong Island, these reservoir hosts are capable of maintaining transmission in the population, even if perfect sanitation were to be achieved for all humans. Therefore, although improved sanitation and mass drug administration substantially reduce the mean worm burden in humans, additional strategies, such as behavioral changes in the feeding practices of domestic pets, safe fish production and/or snail control, would be necessary to eliminate *O. viverrini* transmission in Khong Island.

1819

OUTCOME OF TWO PHASE I RELATIVE BIOAVAILABILITY STUDIES IN HEALTHY VOLUNTEERS AFTER ADMINISTRATION OF THE NEW PEDIATRIC ODT FORMULATIONS OF RACEMATE PRAZIQUANTEL (RAC-PZQ) AND OF THE ACTIVE ENANTIOMER OF PRAZIQUANTEL (L-PZQ)

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Praziquantel (PZQ) was developed in the 1970s to treat schistosomiasis. PZQ tablets are available to treat adults and school aged children, but there is a pressing need to develop a suitable pediatric formulation for treating preschool children. New pediatric oral disintegrating tablets (ODTs) of racemic Praziquantel (rac-PZQ), as well as of the active L-enantiomer of PZQ (L-PZQ), are under development by the Pediatric Praziquantel Consortium. These ODT formulations were assessed for their relative bioavailability against the reference PZQ tablets (Cysticide) in 2 randomized cross-over studies in healthy males. Each study included resp. 32 and 36 subjects, who received single oral doses dispersed in water (ODT formulation) or as tablets (Cysticide), with a wash-out of 7 days in between. Treatments were resp. rac-PZQ ODT at oral doses of 20, 40 and 60 mg/kg and L-PZQ ODT at doses of 10, 20 or 30 mg/kg under fed conditions, and either 40 (rac-PZQ) or 20 (L-PZQ) mg/kg ODT under fasting conditions, and 40 mg/kg PZQ (Cysticide) under fed conditions. Plasma samples for PK were taken at pre-specified time-points up to 24 hours and concentrations of L- and D-PZQ were measured with a validated enantioselective LC-MS/MS method. PK parameters Cmax and area under the curve (AUC) were calculated. After administration of L-PZQ no conversion to the D-PZQ enantiomer was seen. PK profiles after administration of all formulations were quite variable, showing a food effect and supra-proportionality not allowing to build a compartmental model describing the PK profiles. Instead, a linear mixed effects model was built to describe the PK parameters Cmax and AUC and used to predict the dose-exposure relationship in children. Exposure to L-PZQ administered as rac-PZQ ODT or as Cysticide tablets was comparable, but both were higher than after administration of equivalent doses of L-PZQ as ODT. The low exposure (40%) of L-PZQ after administration of L-PZQ ODT compared to administration of equivalent amounts of racemic-PZQ tablets indicates the need for higher dosages of L-PZQ ODT to be administered to achieve therapeutic effects.

1820

COMPARATIVE EFFICIENCY OF *BIOMPHALARIA PFEIFFERI* AND B. *SUDANICA* AS INTERMEDIATE HOST SNAILS FOR *SCHISTOSOMA MANSONI* AND ITS IMPLICATIONS FOR TRANSMISSION OF SCHISTOSOMIASIS IN KENYA

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In Kenya, schistosomiasis infects an estimated 6 million people with >30 million people at risk of infection. *Schistosoma mansoni* is commonly transmitted by *Biomphalaria pfeifferi*, an inhabitant of streams and small water bodies, and *B. sudanica*, which is mostly found along shores of Lake Victoria. Recent studies have accentuated the role of infected snails in maintaining transmission as some snails can survive for over a year shedding cercariae daily. We sought to determine if these two snail species may differ with respect to the efficiency with which they support *S. mansoni* infections. We exposed field-derived *B. pfeifferi* (Kirinyaga, central Kenya) and *B. sudanica* (Kisumu, western Kenya) to *S. mansoni* derived from human subjects from Kirinyaga or Kisumu. The reciprocal cross infection design allowed us to ascertain if local adaptation effects might influence infection outcomes. Juvenile (<6 mm shell diameter), young adult (6-9 mm) and adult snails (> 9 mm) were exposed, all to one miracidium/snail. Overall, *B. pfeifferi* consistently had higher infection rates than *B. sudanica* (39.6 - 80.7% vs. 2.4 - 21.5%), regardless of the source of *S. mansoni* or the size of the snails used. Allopatric *B. pfeifferi* - *S. mansoni* combinations had higher infection rates than sympatric combinations while *B. sudanica* showed the opposite trend. Infection rates were inversely proportional to snail size. Mean daily cercariae production was greater for *B. pfeifferi* exposed to sympatric than allopatric *S. mansoni* (62 –2465 and 100 – 1232, respectively), and this trend increased with snail size. Overall mean daily cercariae production amongst all *B. sudanica* was low (50–590) with no significant differences between sympatric or allopatric combinations, or among the different snail sizes ($p < 0.05$). In conclusion *B. pfeifferi* is more likely to become infected and to shed more cercariae than *B. sudanica,* suggesting that the per snail risk of perpetuating transmission in Kenyan streams and lacustrine habitats may differ considerably with noteworthy implications for understanding transmission dynamics and planning control efforts.

1821

DEVELOPMENT OF A NONHUMAN PRIMATE MODEL OF ZIKA VIRUS INFECTION IN PREGNANT AND NON-PREGNANT RHESUS MACAQUES

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Zika virus has recently been identified as the cause of clinically significant disease with outcomes including fetal abnormalities in the Americas. However, little is known about the natural history of Zika virus, nor the full spectrum of associated diseases. To investigate virus dynamics and immune responses *in vivo*, we developed a rhesus macaque model for Zika virus infection. We also examined the effects of maternal Zika virus infection on fetal development at different stages of pregnancy. We subcutaneously inoculated non-pregnant and pregnant animals with Asian or African lineage Zika virus. Viral RNA was detected in plasma one day post-infection (dpi) in all animals, with peak viral loads reaching above 1 x 10^5 viral RNA copies/mL. Viral RNA was also present in saliva, urine, and cerebrospinal fluid, consistent with case reports from infected humans. Two of four pregnant animals remained viremic for longer periods than non-pregnant animals. Viral RNA was detected in amniotic fluid in one pregnant animal infected during the third trimester. In all animals, infection was associated with transient increases in proliferating natural killer cells, CD8+ T cells, CD4+ T cells, and plasmablasts. Neutralizing antibodies were detected in all animals by 21 dpi. Rechallenge of non-pregnant animals with the Asian lineage Zika virus resulted in no detectable virus replication, suggesting that primary Zika virus infection elicits protective immunity against homologous and heterologous virus strains. Measurements of fetal growth by ultrasonography, examination of fetal brain abnormalities by magnetic resonance imaging, and tissue tropism studies in fetuses are ongoing. These studies establish that Asian lineage Zika virus infection of rhesus macaques provides a relevant animal model for studying natural history and pathogenesis in pregnant and non-pregnant nonhuman primates.

1822

ZIKA VIRUS INFECTION OF HUMAN PLACENTAL CELLS AND EXPLANTS: THE ROLE OF ZIKV RECEPTORS AND ANTI-FLAVIVIRUS ANTIBODIES

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The Zika epidemic that began in Brazil and spread throughout the Americas has been reported as definitively linked to severe birth defects - microcephaly, miscarriage and stillbirth. Detection of ZIKV RNA in the placenta and fetus as well as intrauterine growth restriction suggests extensive infection of the placenta leading to substantial virus-induced pathology. Our studies in placental explants and primary cells isolated from human placenta reveal that prototype and recently isolated Nicaraguan ZIKV 2016 strains infect cells that express AXL, Tyro3 and TIM1 tyrosine kinase receptors, which mediate infection by ZIKV and the closely related dengue virus (DENV) in skin. Infected placental cells, including fetal amniotic epithelial cells, placental fibroblasts, umbilical vein endothelial cells and trophoblast progenitor cells (TBPC), developed cytopathology and expressed ZIKV envelope and nonstructural NS3 proteins, and virus titers released depended on receptors expressed and gestational age. Indicative of infection route, AXL was detected in decidua (uterine decidual cells, invasive cytotrophoblasts), chorionic villi (placental fibroblasts, Hofbauer cells, blood vessels) and fetal membranes (amniotic epithelial cells, TBPC). ZIKV-infected cells downregulated AXL, which was strongly induced in neighboring cells, suggesting a contribution to infection. Differential expression of receptors suggests how ZIKV could infect the decidua and spread to the placenta, fetus and amnion-chorionic membranes. Further, in endemic regions, cross-reactive pre-existing antibodies to DENV could play a critical role in protection or pathogenesis of ZIKV in placenta tissues during pregnancy. Thus, we are studying the neutralizing and potentially enhancing role of ZIKV-specific and DENV-cross-reactive antibodies on infection in primary placental cells and explants. These studies reveal molecular mechanisms of ZIKV infection and routes of virus transmission to the fetus, and we are using the model to assess the therapeutic potential of antibodies and small molecule inhibitors to block infection and prevent congenital disease.

1823

THE CRYO-EM STRUCTURE OF ZIKA VIRUS

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Zika virus (ZIKV) has recently attracted global notoriety due to its explosive spread through the Pacific Islands and Latin/Central America and its link to neurological complications such as congenital microcephaly and Guillain Barré syndrome. It poses a looming threat to many countries (including USA) infested with the *Aedes* species of mosquitoes that transmit the virus. ZIKV can also be sexually and vertically transmitted which expands the geographical reach of the virus. The World Health Organization therefore declared Zika epidemic as 'a public health emergency of international concern'. ZIKV is a positive sense RNA virus belonging to the Flaviviridae family that includes other pathogenic viruses such as dengue virus, West Nile virus, yellow fever virus and tick-borne encephalitis virus. An understanding of the biology of the virus and the mechanism of disease are required to provide appropriate recommendations to tackle the virus and for timely development of diagnostic kits, vaccines and antivirals. We have determined the structure of mature ZIKV at 3.8Å resolution using cryo-electron microscopy The structure of ZIKV is similar to other known flavivirus structures except for the ~10 amino acids that surround the Asn154 glycosylation site found in each of the 180 surface envelope glycoproteins that make up the icosahedral shell. The carbohydrate moiety associated with this residue may function as an attachment site of the virus to host cells. This region varies not only among ZIKV strains but also in other flaviviruses and suggests that changes in this region could influence virus transmission and disease. The atomic structure of ZIKV and its comparison with other flaviviruses will be discussed.

1824

BOOSTING ALTERS THE CROSS-NEUTRALIZING CAPACITY OF ANTIBODY-RESPONSE FOLLOWING ZIKA EXPOSURE IN C57BL/6 MICE

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Zika virus (ZIKV) has recently emerged in the Americas, in areas where the related Flavivirus, dengue virus (DENV), is already endemic. Cross-neutralization of antibodies among Flaviviruses has previously been demonstrated, and reports from diagnostic serological tests have suggested this cross-reactivity occurs among DENV1-4 and ZIKV. In this study, we investigate the cross-neutralizing capacity of the antibody population after subcutaneous exposure to ZIKV in C57BL/6 mice using plaque reduction neutralization tests (PRNTs). Exposed mice initially produced very highly neutralizing antibody to ZIKV (PRNT80) that also highly cross-neutralized DENV2 (PRNT50). After a regimen of homologous boosting with ZIKV, the antibody neutralization capacity remained very high to ZIKV (PRNT80). However, the cross-neutralization to DENV2 decreased to almost nothing. These results indicate that C57BL/6 mice produce a strong antibody response in the absence of robust viremia, suggesting its utility as a model for investigating antibody responses to ZIKV and cross-neutralization to at least DENV2. These results further suggest that homologous boosting with ZIKV contributes to the evolving specificity of the antibody population toward ZIKV in these mice, as it decreases cross-neutralization of heterologous Flaviviruses.

1825

VECTOR COMPETENCE OF AMERICAN MOSQUITOES FOR MULTIPLE STRAINS OF ZIKV REPRESENTING EACH GENETIC CLADE

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In 2015 Zika virus (ZIKV; Flaviviridae. Flavivirus) emerged in the Americas, causing millions of infections in dozens of countries from Brazil to Mexico. The rapid spread of the virus and the association with concerning disease outcomes such as Guillain-Barré syndrome and microcephaly make understanding transmission dynamics essential. Currently, there are no reports of vector competence (VC) of American mosquitoes for ZIKV isolates from the Americas. Further, it is not clear whether locally circulating strains display enhanced transmissibility by local mosquitoes. First, we determined if/whether freezing ZIKV prior to experiments impacts VC estimates as has been shown for dengue virus. Mexican *Aedes aegypti* mosquitoes were given an infectious bloodmeal with either fresh or frozen ZIKV that was originally isolated from an infected human in Puerto Rico (Strain PRVABC59, Asian clade). While infection and transmission rates were significantly higher in mosquitoes fed fresh virus on day 7 post-exposure, no differences were observed in infection, dissemination or transmission rates by day 14, and high infection rates were observed, indicating that previously frozen virus could be used for ZIKV vector competence studies. *Ae. aegypti* mosquitoes were then infected with viruses from the other two recognized ZIKV clades, strain 41525 from the West African clade and strain MR766 from the East African clade along with the aforementioned PRVABC59 strain. Studies showed that while

mosquito infection and dissemination rates were different between the three strains, all viruses were able to infect, disseminate, and were found in saliva (all groups greater than 60% transmission rate by 14 days post-exposure) in the American mosquitoes tested, indicating transmission potential. These data demonstrate that American mosquitoes are highly competent for ZIKV from all three viral clades and that emergence of viral strains from Africa in addition to the currently circulating Asian lineage strain should be monitored.

1826

MAPPING ZIKA VIRUS CROSS-NEUTRALIZING EPITOPES

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The recent emergence and spread of Zika virus (ZIKV) in the Americas has presented a global Public Health emergency and new therapeutic strategies are needed to protect against severe life threatening infections. Moreover, several groups have identified a strong causal relationship between prenatal ZIKV infection and microcephaly in the developing fetus, as well as an association with other serious brain abnormalities. While the ZIKV adult pathologies are less severe than those caused by other flaviviruses, immunity in pregnant females may offer protection from the more devastating outcomes in infected fetuses. ZIKV is closely related to dengue viruses. Moreover, the close phylogenetic relationship between ZIKV to DENV provides an opportunity study the antigenic relationships between these two flavivirus strains. Using a panel of human and mouse monoclonal antibodies generated against various dengue virus (DENV) strains, we have identified some with pan-flavivirus binding reactivity. Using an *in vitro* neutralization assay, a binding assay, and an *in vivo* protection study, we have shown that many DENV antibodies bind to ZIKV, but only a few were broadly neutralizing and mapped onto the E glycoprotein dimer. As structural studies indicate that ZIKV is very mature, it was also not surprising that the prM targeting antibodies did not bind ZIKV. These experiments have revealed a set of monoclonal antibodies targeting a highly conserved neutralizing epitope in DENV and ZIKV. Using an *in vivo* mouse model, we are currently testing the ability of these broadly cross neutralizing antibodies to protect against ZIKV virus infection, potentially identifying a therapeutic antibody for human use.

1827

DEVELOPMENT AND CHARACTERIZATION OF LIVE ATTENUATED VACCINE CANDIDATES FOR ZIKA VIRUS

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The goal of the NIH Laboratory of Infectious Diseases (LID) vaccine program is the development of market-sustainable, live-attenuated vaccines for several medically- important flaviviruses. In the past, the focus has been on the development of the tetravalent dengue virus (DENV) vaccine, which is currently undergoing Phase III evaluation by the Butantan Institute in Brazil. With the recent outbreak of Zika virus (ZIKV) in Latin America, attention has been turned to leveraging the dengue vaccine platform for the creation of vaccine candidates against ZIKV. The live vaccine candidates should be minimally reactogenic, highly immunogenic across all age-groups, cost-effective, and safe for the community. In addition, it would be ideal if they were compatible with the existing tetravalent dengue formulation to allow for inclusion into a pentavalent dengue/Zika formulation for use in regions where these viruses co-circulate. Recombinant chimeric viruses expressing the structural proteins of ZIKV in the background of different DENV serotypes containing the delta-30 deletion have been generated. In addition, full-length ZIKV cDNA molecules containing altered 3' untranslated regions are also under construction. Preclinical trials in mice and rhesus monkeys are being used to demonstrate the attenuation phenotype of these ZIKV candidates and to down-select suitable strains. Phase I trials will evaluate the safety and immunogenicity of both monovalent ZIKV vaccine candidates and combinations with the tetravalent DENV vaccine. The LID is also developing a human challenge model for ZIKV using cGMP isolates or recombinant-derived strains. Such a model can be used for investigating vaccine-induced protection, establishing immune correlates of protection, and to accelerate a possible regulatory pathway toward licensure in the face of decreased transmission at the time of future Phase III efficacy trials. The challenge model will also be used to facilitate studies of ZIKV viremia in populations with well-defined pre-existing antibody profiles and to quantify the duration and level of ZIKV shedding in body fluids other than serum.

1828

THE ROLE OF ECDYSONE RECEPTOR IN *ANOPHELES GAMBIAE* MOSQUITO POST-MATING BIOLOGY

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The *Anopheles gambiae* mosquito is responsible for infecting millions of people with malaria each year throughout Africa. Female Anophelines mate a single time in their life making reproduction a crucial point in their life cycle, and a potential target for vector control. The critical insect steroid hormone, 20-hydroxyecdysone (20E), is essential for regulating larval development and egg production in numerous insect species. Recently, our lab identified multiple novel roles for 20E in *An. gambiae* reproduction. We demonstrated that sexual transfer of 20E during copulation is necessary and sufficient to induce two key female post-mating phenotypes: oviposition and refractoriness to multiple matings. Here we show that male-transferred 20E induces these phenotypes by initiating signaling cascades following its interaction with specific receptors localized to the female reproductive tissues. Ecdysone Receptor (EcR) is known to be an ecdysone-responsive nuclear receptor regulating 20E signaling during larval development, metamorphosis, and adult female vitellogenesis. Our findings suggest that EcR in *An. gambiae* is responsible for regulating 20E-induced oviposition, while female mating refractoriness is induced through a novel, yet unidentified, 20E receptor. We have also discovered that in an EcR depleted background, females fail to store sperm. This sensitized genetic background can be used to provide important biological insights into the mechanism of sperm storage - a critical process for the female's lifelong fertility. Overall, understanding the mechanisms through which male-transferred 20E induces vast transcriptional and physiological changes in the female *An. gambiae* mosquito can not only advance knowledge of unique vector reproductive biology, but it can also reveal novel biological targets for mosquito control.

1829

PLASMODIUM FALCIPARUM PFS47 GENETIC DIVERSITY IN FIELD COLLECTED *ANOPHELES GAMBIAE* AND *ANOPHELES COLUZZI* FROM MALI, AFRICA

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The anopheline immune system has the capacity to mount effective antiplasmodial responses. We have shown previously that Pfs47 is required by *Plasmodium falciparum* to evade the *Anopheles* immune system, which can be an important barrier for the adaptation of the parasite to different vectors. Adaptation of *P. falciparum* to evolutionary distant anophelines appears to involve natural selection of compatible Pfs47 haplotypes. Pfs47 presents high genetic diversity in Africa and strong geographic structure at

continental level worldwide, consistent with natural selection of the gene by different anophelines. Here we test whether Pfs47 may be differentially selected by sympatric African malaria vectors. We studied Pfs47 genetic diversity being transmitted by *An. gambiae* and *An. coluzzi* in a small region of Mali. Pfs47 was genotyped in a total of 150 sporozoite-infected mosquitoes collected throughout the year. Multiple Pfs47 haplotypes were detected in 26% of the mosquitoes. A high diversity of Pfs47 was detected (11 haplotypes) with 2 haplotypes accounting for 73% of the *P. falciparum* infected mosquitoes. Temporal analysis of haplotype distribution showed Pfs47 haplotype present diversity throughout the year in both species of mosquitoes. The most frequent Pfs47 haplotypes were present in both *An. gambiae* and *An. coluzzi*, but there were some differences in the frequency between both species. *An. gambiae* and *An. coluzzi* don't appear to be genetically distant to cause major differential selection of Pfs47 haplotypes.

1830

HYBRID ALLELIC IMBALANCE AND GENE EXPRESSION EVOLUTION IN THE *ANOPHELES GAMBIAE* SPECIES COMPLEX

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The accumulation of genetic incompatibilities that cause hybrid sterility and/or inviability between diverged populations is an important step in the formation and maintenance of species boundaries in the face of hybridization. In the *Anopheles gambiae* species complex, F1 hybrid males are sterile, while females are fully fertile and can backcross to either parental species. Thus, F1 hybrid females facilitate the introgression of genomic regions between species (e.g. chromosomal inversions) that may be adaptive. While some chromosomal incompatibilities that cause hybrid sterility and inviability between *An. gambiae* and *An. arabiensis* have been identified, little is understood about the nature of these incompatibilities. Divergence in gene expression, rather than genetic differences, is thought to account for a large proportion of phenotypic differences between species, and may also play a role in hybrid sterility and inviability. We analyzed gene expression in F1 hybrid male and female pupae and compared it to parental males and females in bi-directional crosses between *An. gambiae, An. arabiensis,* and *An. quadriannulatus*. By analyzing genome-wide, allele-specific gene expression, we explored the roles of *cis* and *trans* regulatory divergence between species and in male and female hybrid phenotypes. The relationship between allele-specific expression, patterns of sequence evolution, and known hybrid sterility/inviability QTL was also explored. Our analysis provides insight into gene expression divergence and evolution in the *An. gambiae* species complex.

1831

HEMOCYTE-SPECIFIC MANIPULATION OF THE IMD PATHWAY AFFECTS *PLASMODIUM* INFECTION IN *ANOPHELES STEPHENSI*

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Using a newly developed hemocyte specific *Gal4*/UAS-based expression system in *Anopheles stephensi* we have begun to investigate the role of the IMD pathway in the hemocyte's ability to respond to a *Plasmodium* infection. The IMD pathway has been shown to contribute to the mosquito's ability to fight a *Plasmodium* infection through studies using RNAi as a method to perturb post-transcriptional gene expression in the IMD pathway. However, due to the pleotropic effects of dsRNA injections it becomes difficult to ascertain the individual contributions of various tissues to the immune response against a *Plasmodium* infection beyond the midgut stage of parasite development. Using the *Gal4*/UAS system we are able to disrupt the IMD pathway specifically in hemocytes, the cellular component of the mosquito's innate immune system, and analyze the mosquito's response to a parasite challenge. We have found that manipulation of the IMD pathway by knocking down or over expressing *Caspar* leads to altered levels of infection in both midguts and salivary glands in comparison to controls. This suggests the IMD pathway plays an important role in the hemocyte's ability to defend against infection during multiple stages of *Plasmodium* infection, independent of a normal IMD pathway in all other tissues. This approach allows for questions to be answered concerning the parasite-vector interaction during the post-midgut stage of parasites development; a pivotal point in defense against salivary gland invasion by sporozoites.

1832

LANDSCAPE GENETICS OF PYRETHROID RESISTANCE IN *ANOPHELES ARABIENSIS* IN KENYA

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Anopheles arabiensis have become increasingly abundant in Africa and are playing an important role in maintaining residual malaria transmission in sub-Saharan Africa. Overall, insecticide resistance in *An. arabiensis* has remained relatively low with respect to *An. gambiae*. However, recent studies suggest that resistance in *An. arabiensis* is emerging in Africa. Resistance could potentially increase and spread rapidly if gene flow between populations is large. Knowledge of *An. arabiensis* population genetic structure is critical to understanding insecticide resistance spread. We test how various ecological variables affect gene flow (dispersal) using a landscape genetics approach utilizing techniques from population genetics, landscape ecology, and spatial statistics. We genotyped *An. arabiensis* collected from 14 study sites across Kenya at 10 microsatellite loci and at kdr L1014F/S. We created resistance surfaces in ArcGIS for key environmental and landscape variables hypothesized to influence gene flow of *An. arabiensis*. We optimized resistance surfaces using the ResistanceGA package in R which utilizes a genetic algorithm to optimize surfaces based on pairwise genetic distances and CIRCUITSCAPE resistance distances. Lastly, mixed effects models were fit by maximum likelihood in the lme4 package in R. We observed both kdr 1014F and 1014S alleles. Kdr mutation frequencies were 0.023 and 0.103 at two sites in western Kenya and were absent from other sites. We hypothesize that forest cover and elevation provide the greatest barriers to gene flow and population density and roads largely promote gene flow. Understanding the factors promoting gene flow and insecticide resistance spread is critical to informing antimalarial interventions, especially since pyrethroid resistance in *An. arabiensis* is relatively low and patchily distributed.

1833

GENOMIC ANALYSIS OF THE *ANOPHELES GAMBIAE* BAMAKO ECOTYPE

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The Bamako ecotype is a chromosomal form of *Anopheles gambiae* found in southern Mali and northern Guinea in association with rock pools in the Niger River. The ecotype is defined by three fixed chromosomal inversions on arm 2R, *j*, *c*, and *u*, the latter two of which also segregate at appreciable frequencies in sympatric *An. coluzzii* and non-Bamako *An. gambiae*. Previous studies have found evidence for some degree of assortative mating within the Bamako ecotype, but the status of this ecotype as an independently evolving entity remains unclear, as does the genomic basis of differential habitat preferences between Bamako and non-Bamako forms. Clarifying the status of this ecotype offers a chance to explore the genomic basis of habitat adaptation in *An. gambiae*. Using

pooled resequencing (*pool-seq*) of Bamako, non-Bamako *An. gambiae*, and *An. coluzzii* from southern Mali, as well as individual whole-genome resequencing, we present the first whole-genome analysis of the Bamako chromosomal form. Clustering of individual samples provides evidence for Bamako as an independent entity, while genome scans show that differentiation between Bamako and other sympatric populations is concentrated in the inversions that define Bamako. However, the strongest signals of differentiation are found not in the 2R*j* inversion, which is relatively unique to Bamako in this geographic region, but in inversions 2Rc and *u*, which are also found in sympatric populations of non-Bamako *An. gambiae* and *An. coluzzii*. This pattern of differentiation in shared inversions is partially driven by novel, Bamako-specific alleles in genes known to be involved in insecticide resistance, which may be candidate genes for habitat adaptation in this ecotype.

1834

ASSESSMENT OF THE POST-ZYGOTIC REPRODUCTIVE BARRIERS BETWEEN *ANOPHELES GAMBIAE* ET *AN. COLUZZI*I

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Anopheles gambiae and *An. coluzzi*i are two of the most important malaria vector species in sub-Saharan Africa. These recently-diverged sibling species are thought to be separated by strong assortative mating combined with selection against hybrids. At present, little is known about hybridization and the post-zygotic reproductive barriers between these cryptic taxa. Swarm segregation and assortative mating between *An. gambiae* and *An. coluzzi*i were studied in the villages of VK7 and Soumousso, Western Burkina Faso. Natural swarms and pairs in copula were collected and genotyped, the proportion of intra and interspecific matings determined, and interspecific sperm transfer checked genetically. Females were collected resting indoors or as larvae and genotyped or sexed-and-genotyped via a multiplex PCR. Larval development and adult swarming success of hybrids were also estimated and compared to the parental species in semi-field experiment. A total of 3,687 males and 220 females were collected from 109 natural swarms and genotyped. Amongst 187 females captured in copula, 4 *An. gambiae* and one *An. coluzzi*i females were found paired with and inseminated by heterospecific males. The lower overall hybridization rates observed at the larval and adult indoor stages compared to cross-mating rates support post-mating selection processes acting against hybrids. A total of 5,400 first instar larvae were transplanted in 36 cages in rice field with or without predators. Although no statistical difference was found between reciprocal hybrids and parental species in adult wing size, the development sucess varied significantly. In total 6,400 males of *An. gambiae*, *An. coluzzi*i and the two reciprocal hybrids were randomly released in grand cages and swarm activities were daily monitored. A total of 428 males were captured in swarms but the frequencies of *An. coluzzi*i (61.68%) followed by *An. gambiae* (37.62%) were significantecly higher than those of the hybrids (0.70%). These findings are important for our understanding of the process of sympatric speciation in these important vector species.

1835

INTEGRATED PEDIATRIC FEVER MANAGEMENT AND ANTIBIOTIC OVER-TREATMENT IN MALAWI HEALTH FACILITIES: DATA MINING A NATIONAL FACILITY CENSUS

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There are concerns about growing irrational antibiotic prescription practices in the era of test-based malaria case management. This study assessed integrated pediatric fever management using malaria rapid diagnostic tests (RDT) and Integrated Management of Childhood Illness (IMCI) guidelines, including the relationship between RDT-negative results and antibiotic over-treatment in Malawi health facilities in 2013-2014. A Malawi national facility census included 1,981 observed sick children 2-59 months with fever complaints. Weighted frequencies were tabulated for other complaints, assessments, and prescriptions for RDT-confirmed malaria, IMCI-classified pneumonia, and clinical diarrhea. Classification trees using model-based recursive partitioning estimated the association between RDT results and antibiotic over-treatment and learned the influence of 38 other input variables at patient-, provider-, and facility-levels. Among 1,981 clients, 72% were tested or referred for malaria diagnosis and 85% with RDT-confirmed malaria were prescribed first-line anti-malarials. 28% with IMCI-pneumonia were not prescribed antibiotics (under-treatment) and 59% 'without antibiotic need' were prescribed antibiotics (over-treatment). Few clients had respiratory rates counted to identify antibiotic need for IMCI-pneumonia (18%). RDT-negative children had 16.8 (95% CI: 8.6-32.7) times higher antibiotic over-treatment odds compared to RDT-positive cases conditioned by cough or difficult breathing complaints. Integrated pediatric fever management was sub-optimal for completed assessments and antibiotic targeting despite common compliance to malaria treatment guidelines. RDT-negative results were strongly associated with antibiotic over-treatment conditioned by cough or difficult breathing complaints. A shift from malaria-focused 'test and treat' strategies toward 'IMCI with testing' is needed to improve quality fever care and rational use of both anti-malarials and antibiotics in line with recent global commitments to combat resistance.

1836

VALIDATION OF MATERNAL RECALL OF CARE-SEEKING EVENTS FOR CHILDHOOD ILLNESS IN SOUTHERN PROVINCE, ZAMBIA

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Seeking care from an appropriate provider is the first step in accessing correct management of an episode of childhood illness. Accuracy of maternal-reported care-seeking timing and source of care as collected through household surveys has not been validated in sub-Saharan Africa. A 2016 survey compared reported care-seeking against a gold-standard of documented care-seeking events among a random sample of mothers of children <5 years old in Southern Province, Zambia. A total of 1,156 enrolled children were assigned cards with unique barcodes. A total of 75 potential providers of child curative services in the study area participated in care-seeking event tracking. Providers were given smartphones with

a barcode reader and instructed to scan the cards of all children seeking care at the source, generating an electronic record of the care-seeking event. Additionally, providers gave all caregivers accessing care for a child <5 provider-specific tokens used to verify the point of care during the household survey. Reported care-seeking events were ascertained in each household using a questionnaire modeled off the Zambia Demographic and Health Survey (ZDHS). The ZDHS defines childhood illness as fever, cough with rapid breathing, and/or diarrhea in a child under 60 months of age in the two weeks preceding the survey. Recall of care-seeking events for childhood illnesses reported by mothers was compared against the gold-standard documented care-seeking events to estimate the accuracy of maternal recall of care-seeking behavior. Care-seeking data were collected for 537 children in urban areas and 547 children in rural areas. We present findings on the accuracy, sensitivity, and positive predictive value of caregiver report of care-seeking location by key socio-demographic characteristics. This study assesses whether the current standard care-seeking indicator measured through household surveys can produce valid estimates of care-seeking for childhood illness. This will be used to determine whether new methods are needed to estimate care-seeking behavior to measure progress in global investments in child survival interventions.

1837

POLYPHARMACY, TREATMENT SEEKING, AND DIAGNOSTIC TESTING IN A POPULATION-BASED SURVEY OF FEBRILE ILLNESS IN WESTERN KENYA

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Many fever episodes in malaria endemic areas are treated in the informal sector. Minimal access to diagnostic testing, both in the formal and the informal sectors, lends itself to the potential for polypharmacy and other forms of inappropriate use of medicines. We describe the type and the number of medicines consumed for a febrile illness in a community survey conducted as part of a larger study. Data on any laboratory tests done for the fevers were also collected. The study population consisted of household members above one year of age with a history of a febrile illness in the preceding one month. Out of the 2,007 clients reporting a history of fever for which they took an action, 99.1% reported taking medication for the fever, mainly antipyretics/analgesics (86.7%) and antimalarials (76.4%). Use of antibiotics was reported at 29.8%. Forty seven percent of patients who took a medicine took two different drugs; the commonest combination being an antimalarial and an analgesic. Twenty eight percent (28%) took three different medications while nearly 10% reported taking four or more medicines. Only 15.4% reported using a single drug. The majority of those who took an antimalarial (72.7%) reported using an ACT while the rest received either Sulphadoxine Pyremethamine (SP) or quinine. A malaria test was performed on 44.3% of the clients, while in 229 cases (11.4%) a test other than a malaria test was performed, most commonly for typhoid fever (81.7%), and brucellosis (18.8%). This was in addition to a malaria test for 221 (96.5%) of the 229 for whom a non-malaria test was performed. Twelve percent reported being tested for both typhoid fever and brucellosis. Clients above 5 years of age, those who had a laboratory test, and those visiting a drug shop for fever management were more likely to receive more than two drugs ($p<0.001$). Clients under 5 years were more likely to have a malaria test done than those aged > 5 ($p<0.001$). There is a high rate of consumption of multiple drugs for fever which is exacerbated by poor access to diagnostic testing. There is need for strategies to promote evidence-based management of fevers and rational use of drugs in the community.

1838

QUALITY IMPROVEMENT STRATEGIES TO MONITOR CHVS MRDT PERFORMANCE: A CASE OF MALARIA TESTING IN WESTERN KENYA

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Use of Community Health Workers (CHWs) in malaria diagnosis has been recommended as a task-shifting strategy to counter the shortage of health workers in resource-limited settings. However, monitoring their performance is a challenge. We implemented a set of strategies for monitoring CHW performance of malaria rapid diagnostic tests (mRDTs): 1) Post-training evaluation, 2) regular supervision and refresher training visits, and 3) monitoring of error rates. In the context of a larger study, we trained 300 CHVs from 16 Community Units (CUs) in two sub counties in western Kenya on proper use of mRDTs. At the conclusion of training, we measured CHW proficiency with a standardized mRDT checklist and evaluated their interpretation of a set of used mRDTs. CHWs then administered and interpreted mRDTs within their CUs to any patients with symptoms and/or reported history of fever. Clients with a positive mRDT received an antimalarial discount redeemable at selected private medicine outlets when presented along with their positive mRDT cassette. We collected mRDTs interpreted as positive from the outlets, and mRDTs interpreted as negative from the CHWs. We then re-interpreted the mRDTs to confirm results and test quality. In the first 6 months, we convened a total of 10 supervision meetings with each CHW group in the 16 CUs, and provided refresher training as necessary. After 6 months, 10,872 clients had been tested, 2,256 (19.96%) of which had a positive mRDT and 8616 (80.04%) of which had a negative mRDT. The total mRDT errors were 138 tests (1.27%), with 62 (3%) false positive and 76 (1%) false negative. The incidences of false positive and false negative results trended downwards as supervision visits continued over time; month 1-2: 14.5/23, month 3-4: 10.5/11, month 5-6: 6/3.5. The findings suggest that coupling supervision meetings with confirmation of mRDT results can help identify errors and refresher training needs, as well as improve CHW performance in accurate malaria diagnosis.

1839

MISSED OPPORTUNITIES FOR INTERMITTENT PREVENTIVE TREATMENT IN PREGNANCY FOR MALARIA: EVIDENCE FROM THE KENYA DEMOGRAPHIC AND HEALTH SURVEY, 2014

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Malaria in pregnancy (MIP) is associated with maternal anemia, placental parasitemia, low birth weight and increased perinatal mortality. Intermittent preventive treatment in pregnancy (IPTp) with sulphadoxine-pyrimethamine (SP) is recommended to reduce MIP-associated risk in medium-to-high malaria transmission areas. In Kenya, although antenatal care (ANC) clinic attendance is high, the proportion of women receiving at least two doses of IPTp-SP has historically been low. We assessed the factors associated with missed IPTp opportunities during pregnancy. We analyzed data from the 2014 Kenya Demographic and Health Survey, a two-stage cluster sample, cross-sectional survey of 36,430 households. Missed IPTp opportunities were was defined as a woman aged 15–49 years who attended at least four ANC visits and lived in the 14 malaria-endemic counties with IPTp policy but received fewer than two doses of IPTp-SP during their last completed pregnancy. We used logistic

regression to compare missed IPTp-SP opportunities with demographic, socio-economic and geographic factors. Of the 909 women who attended at least four ANC visits in the 14 malaria-endemic counties, 30.5% (n=277) had a missed opportunity for IPTp during pregnancy. In univariate analysis, living in the lake-endemic region (OR=1.7; 95% CI: 1.1–2.4; p=0.008), parity >4 children (OR=1.5; 95% CI: 1.04–2.22; p=0.028) and more than secondary education (OR=3.5; 95% CI: 1.6–7.5; p=0.001) were significantly associated with missed IPTp opportunities. In multivariate analysis, women with more than secondary education had significantly higher odds (OR=3.3; 95% CI: 1.4–7.9, p=0.007) of missed opportunities for IPTp. Despite high ANC attendance, almost one-third of pregnant women had at least one missed IPTp opportunity. Women with higher education were over three times more likely to have missed IPTp opportunities, which might be due to lack of perceived risk by both women and healthcare providers. Studies are needed to identify modifiable factors to increase IPTp uptake among pregnant women.

1840

IMPLEMENTATION OF SEASONAL MALARIA CHEMOPREVENTION IN THE GAMBIA

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Seasonal Malaria Chemoprevention was adopted in the Gambia as a strategy in 2012, included as part of the national policy in 2013, and implemented from 2014. In 2015, an electronic data system was introduced for monitoring delivery, through the ACCESS-SMC project supported by UNITAID. SMC drugs were administered from August to November in four cycles in Upper River Region and Central River Region. SMC was delivered door-to-door. Each child was issued an SMC card bearing a QR code that could be scanned with an android phone each time the child was treated. Information about the child (age, gender and other details) was captured on the phone using iForm, an offline data capture system, and then uploaded to a central database system, eValuate. The system provides information on all the monthly treatments a child has received avoiding the need for registers and allowing timely feedback to the malaria control programme about progress with SMC delivery. At the end of the transmission season, a cluster sample survey was conducted to measure SMC coverage. Communities selected with probability proportional to size, were divided into segments on a sketch map and all the households in one segment, which was chosen at random, were included in the survey. 1174 children under 7 years of age were surveyed, 690 were eligible for 4 SMC cycles and of these, 93% had received an SMC card and at least one SMC treatment. 84% of children had received at least 3 months of SMC treatment. Coverage was lower in the 4th month, which coincided with harvest activities. The main reason for missed doses was being away when the health worker visited. Relatively few children outside the recommended age range were treated, among children 6 to 7 years of age, fewer than 30% had received SMC doses. Door-to-door delivery achieved high coverage of SMC in The Gambia. Outreach strategies may improve coverage at the end of the transmission season.

1841

A CLUSTER RANDOMIZED TRIAL OF TARGETED BEHAVIOR CHANGE COMMUNICATION USING A MOBILE HEALTH PLATFORM TO INCREASE UPTAKE OF LLINS AMONG PREGNANT WOMEN IN TANZANIA: THE HATI-SALAMA PROJECT

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The Hati Salama (HASA) cluster-randomized controlled trial aims to increase malaria awareness among pregnant women using mhealth technology in Tanzania. HASA utilized an electronic system whereby nurses issue vouchers to pregnant women, allowing them to redeem a Long Lasting Insecticidal bednet (LLIN) at a retailer for a highly subsidized cost. A RCT was selected to test efficacy of SMS behaviour change communication messages aimed to increase the uptake of LLINs in areas of Tanzania identified as malaria hotspots with overall low uptake of LLINs. HASA was implemented in 97 antenatal health facilities; 48 clinics were assigned to the control group (no targeted SMS messages sent to beneficiaries) and 49 in the intervention group (targeted messages sent to beneficiaries). In total, 5396 beneficiaries were randomized through cluster randomization of the health center and had LLIN voucher redemption status recorded. There were 2708 beneficiaries from the intervention clinics, and 2688 beneficiaries from the control clinics. There were 25 urban clinics and 23 rural in each arm. The redemption rate was 70.4% in the intervention sites and 67.4% in the control sites. The absolute difference in the redemption rates was 3.5% (95% CI, -3.8% to 11.0%) p=0.35 according to a Rao-Scott estimate stratifying by urban/rural and clustering by clinic. The odds ratio of redemption in the intervention vs. control sites was 1.13 (95% CI, 0.86 to 1.51,p=0.36) according to the GEE method controlling for urban/rural and the prior redemption rate with a working exchangeable correlation to account for the cluster randomized design. The estimated intraclass correlation coefficient (ICC) is 0.11 meaning that 11% of the total variance in the redemption rates was attributable to the clinic and the remaining 89% was attributable to the beneficiary. The use of behavior change communication via SMS had no significant effect on increasing LLIN uptake among pregnant women in this large cluster randomized trial. This suggests that other factors to uptake of LLINs through a voucher program exist. Evaluation of these factors is essential for future implementation of similar programs.

1842

CONCURRENTLY ESTIMATING THE COMPLEXITY OF INFECTION AND SNP ALLELE FREQUENCY FOR MALARIA PARASITES

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Plasmodium falciparum population genetics can inform malaria epidemiology, but a high prevalence of polygenomic infections (those with more than one genotype) can render estimation of even the most basic parameters, such as allele frequencies, challenging. A method, COIL, has been developed to estimate complexity of infection (COI) and allele frequency from SNP data, but relies entirely on monogenomic infections to estimate allele frequencies. However, allele frequency estimates limited to monogenomic infections are biased, and when the average COI is high they can be difficult or impossible to estimate. Here we develop an iterative approach that simultaneously estimates allele frequency and COI from all samples in a population, irrespective of whether they are monogenomic or polygenomic, and uses Markov chain Monte Carlo method to provide Bayesian inference. The method was tested on a series of simulations and then applied to a real dataset from Uganda. We performed Sequenom typing of 105 SNPs in 868 samples from cross-sectional surveys performed in three regions of varying endemicities in Uganda including Walukuba (low-moderate, EIR 2.8), Kihihi (high, EIR 32), and Nagongera (very high, EIR 310). Allele frequencies were used to calculate F_{ST}, a measure of genetic differentiation. Our results suggest high migration rates and little population substructure between the sites (0.016 Kihihi vs. Nagongera, 0.0 for Walukuba vs. Kihihi or Nagongera). Surprisingly, the mean COI in Walukuba (4.7) was similar to Nagongera (4.4) and significantly higher than Kihihi (2.0) despite much lower transmission in Walukuba; this unexpected finding was not explained by parasite density or age. One possible explanation for this finding is that Walukuba is peri-urban setting with a relatively high proportion of cases coming from surrounding regions with higher transmission intensity. This is also consistent with the absence of population structure observed between the other sites. We conclude that this method allows the interpretation of useful population genetic SNP data from polygenomic infections, which are common in high transmission settings.

1843

USE OF SHARED HAPLOTYPES THAT ARE IDENTICAL-BY-DESCENT TO INFER POPULATION STRUCTURE AND PARASITE MIGRATION WITHIN SOUTHEAST ASIA

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Estimates of parasite gene flow may be important in stratifying malaria risk, but to be useful for this purpose those estimates need to reflect contemporary patterns of parasite migration. Haplotypes identical-by-descent are being increasingly used in human genomics for inference of recent demographic events, and can be used to estimate migration rates. Such methods have not been used to infer migration patterns for malaria parasites, and they are just now beginning to be used to document changes in parasite demography as a result of reduced malaria transmission or the rapid spread of drug resistance mutations. The objective of this study is to examine segments of the parasite genome that are identical-by-descent (IBD) to more finely map patterns of parasite population structure and to infer migration patterns at an increasingly local scale. The extent of shared IBD haplotypes was determined by the program Beagle using SNPs genotyped by a *P. falciparum* DNA microarray from samples collected in Southeast Asia and Bangladesh. The extent of IBD sharing was estimated pair-wise between all samples and aggregated both within each study site and between study sites. Preliminary analyses suggest meaningful sharing of IBD haplotypes within study sites, with median IBD segments upwards of 1MB. There is evidence of increased IBD sharing between sites in close geographic proximity, but also some evidence of IBD sharing between more geographically distant sites, which may represent parasite migration through human movement. Patterns of IBD sharing between sites mirror patterns of spreading artemisinin resistance (based on sites sharing K13 haplotypes). IBD sharing based on SNPs from the DNA microarray will be compared to IBD sharing determined from a subset of samples for which whole genome sequences are available. The study of temporal and geographical dynamics of shared IBD haplotypes is a promising approach for delineating contemporary patterns of parasite migration that can be used to identify sources and sinks of malaria transmission.

1844

WHOLE GENOME SEQUENCING USED TO DISTINGUISH *PLASMODIUM VIVAX* RELAPSE FROM REINFECTION AND PRIMAQUINE RESISTANCE IN PERU

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Plasmodium vivax, the most widespread form of malaria, poses a significant challenge to malaria elimination due to its ability to cause relapsed infections from reactivation of hypnozoites. Distinguishing relapses from reinfections or recrudescence is essential for monitoring malaria transmission patterns and detecting anti-relapse therapy resistance. Current methods for genotyping *P. vivax* rely on microsatellite markers, which reveal a limited region of the parasite's genome, making it difficult to differentiate relapses from reinfections. Whole genome sequencing (WGS) of *P. vivax* is a highly sensitive tool for genotyping recurrent infections that has not been widely deployed in field studies. One main reason is because patients with *P. vivax* infections have low parasitemias, so a small amount of human DNA greatly reduces sequencing efficiency. We used a novel technique called selective whole genome amplification (SWGA) to enrich *P. vivax* DNA from whole blood samples. We performed WGS of 81 isolates of *P. vivax* collected from symptomatic patients in Iquitos, Peru during a study to assess three regimens of primaquine. This included 58 paired samples from a person's initial and recurrent infection after primaquine treatment. We obtained high quality sequences with an average coverage of 22x and up to 80% of the genome covered by >5 reads. We identified thousands of high quality single nucleotide polymorphisms, insertions and deletions, and copy number variants, which we will use for further analysis. We will calculate genetic diversity, linkage disequilibrium, complexity of infection, and genes under balancing or directional selection. We will compare paired samples from recurrent infections using a sliding window principle components analysis approach. In this study, we validate a cost-effective and robust method for genotyping *P. vivax* infections that will significantly improve the ability to track *P. vivax* transmission and monitor the efficacy of anti-relapse medication.

1845

A GENOME-WIDE ANALYSIS OF RECENT SELECTION IN AFRICAN MALARIA VECTOR POPULATIONS

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Land use changes, increasing urbanisation and intensification of malaria control programmes are subjecting malaria vectors to a variety of new and intense selective pressures. Here we describe a genome-wide scan for signatures of recent selection in mosquito populations sampled from 8 African countries, using whole genome sequence data from the *Anopheles gambiae* 1000 genomes (Ag1000G) project. We have integrated results from a number of statistical methods, including tests based on haplotype length (iHS, XP-EHH), haplotype diversity (H12), allele frequency spectra (SweeD, delta Tajima's D) and population differentiation (FST, XP-CLR, PBS). This combination of methods allows us to identify selective events that are specific to a single population or shared across multiple populations, to identify both hard and soft selective sweeps, and to find selection events from recent times and the more distant past. As well as validating several previously observed loci, we identify a number of very strong signals of recent selection at novel loci. Of the top 20 strongest previously unseen signals, 10 coincide with metabolic insecticide resistance genes, 2 coincide with with genes that may be involved in cuticle mediated resistance, 1 coincides with genes associated with gustatory behaviour, and 7 have no candidate phenotype. Although we observe a number of hard sweeps, most loci display soft sweeps involving multiple haplotypes. Several loci have come under selection across a broad geographical range, however the pattern of selection is heterogeneous with a number of hits being restricted to a single population or geographical region and some populations showing almost no evidence for recent selection. All data from these analyses will shortly be made publicly available for download and for interactive exploration via a new release of the Ag1000G web application, providing a community resource for further detailed study of selective forces and adaptive responses in natural vector populations.

1846

GENOME-WIDE ASSOCIATION STUDY OF SUSCEPTIBILITY TO SEVERE MALARIA IN 17,500 INDIVIDUALS FROM AFRICA, ASIA AND OCEANIA

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Genome-wide association studies (GWAS) of susceptibility to malaria have until recently been limited by relatively small sample sizes. In addition, the complex pattern of effects observed for some association signals, and poor representation of relevant genetic variation in available reference panels has hampered progress. Here, we undertake a GWAS in 8931 individuals hospitalised with severe malaria and 8703 population controls from 11 malaria-endemic populations, with replication in a further 15,000 individuals. We sequence the genomes of a further 773 individuals from sub-Saharan African populations, and use this data along with publicly available data from the 1000 Genomes Project to accurately impute genotypes genome-wide. We develop methodology to test for association with severe malaria subphenotypes, and identify and replicate a novel locus on chromosome 6 associated with increased risk of cerebral malaria. Across the genome, at least 10 other loci show substantial evidence for association, and we catalogue these in detail. In specific regions, we survey structural variation and use further refined reference panels to fine-map the signal of association, including at the glycophorin region on chromosome 4 where we identify a strong candidate for the functional protective mutation. Our study represents the largest genome-wide study of an infectious disease to date, and will provide an important resource for future studies into the human genetic basis of malarial disease and, potentially, its interaction with parasite processes.

1847

COMPARATIVE TRANSCRIPTOME ANALYSIS OF THE HOST RESPONSE IN BLOOD AND SPLEEN DURING THE COURSE OF A *PLASMODIUM CHABAUDI CHABAUDI* INFECTION

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During the asexual blood stage of malaria in the mammalian host, *Plasmodium* parasites induce alterations in host haematopoiesis, and in the structure and cellular composition of the spleen. Although this organ plays a critical role in generating anti-parasite immunity, spleen samples are generally not accessible in humans; instead, blood samples are typically used to infer the types of immune responses important for controlling infections. Whether the immune signatures identified in the blood are representative of those within the spleen is still largely not known, however. The objective of our study was to compare parasite-induced whole transcriptome changes in blood and spleen using the rodent malaria model of *P. chabaudi chabaudi* in C57BL/6 mice. Specifically we set out to identify common infection signatures, and those that can only be detected in either the blood or the spleen. Infected blood and spleen samples

were collected every 2 days during the acute phase of infection, until day 12 when the parasitaemia was first controlled. Samples collected from naive uninfected mice at day 0 and day 12 were used as controls. Gene expression was quantified using the Illumina mouse WG6 v2 microarray platform (consisting of 45,281 probes sets, representing 30,854 genes). Data were transformed to log fold change with respect to naive controls, and clustering of these short time-series was performed using a Mixtures of Hierarchical Gaussian Processes (MOHGP), which explicitly model the strong time dependency across successive time points. We identified several pathways that are commonly perturbed in blood and spleen, including anaemia, apoptosis and T-cell activation. We also observed that for some genes, expression peaked in the blood before it was measured in the spleen. Importantly a number of pathways, such as erythrocyte production, were identified that were exclusively activated in the spleen but not the blood. We will discuss the implications of these findings for the interpretation of whole blood transcriptome.

1848

MELDING CHEMOGENOMICS AND CHEMOINFORMATICS TO DEFINE MALARIA'S DRUGGABLE GENOME

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With artemisinin resistance (ART-R) spreading in South East Asia the discovery of new drugs to treat malaria is imperative. Thousands of antimalarial compounds have been identified in high-throughput phenotypic screens, but the mode of action (MOA) for most of these compounds is unknown. Efforts to discover the MOA targets of these compounds is hampered since much of the *Plasmodium falciparum* genome is not functionally annotated and therefore deciphering target information for many, if not most, new lead compounds is severely compromised. Combining chemogenomics and chemoinformatics offers the potential to functionally define the druggable genome of *P. falciparum*. Our approach can functionally link unknown genes to more clearly defined genes and GO pathways through the chemogenomic profiles related to the MOA of characterized antimalarial drugs and unknown lead compounds. Importantly, this type of analysis helps to rationally classify leads most likely to be targeting genes that can counter ART-R. More specifically, we are using chemogenomic profiling of isogenic *P. falciparum* single insertion *piggyBac* mutant clones, including several with differential sensitivity to ART. Specific perturbations in metabolic pathways linked to the genetic mutation caused by the *piggyBac* insertion create unique IC_{50} patterns for each compound and similar IC_{50} profiles identify drugs with MOAs likely to be targeting the same pathway. This information can be combined with chemical similarity measures of around 500 prioritized compounds to increase confidence in target prediction and indicate which molecular features are key to the biological response. We identified distinct mechanisms associated with ART sensitivity and resistance in the current screen by RNA-seq of a dysregulated K13 mutant. Chemogenomic and chemoinformatic characterization of malaria inhibiting compounds will help focus the drug discovery agenda on the most effective targets.

1849

HOW PYRETHROIDS RESISTANCE IN *AEDES AEGYPTI* POPULATIONS FROM BRAZIL AFFECTS *WOLBACHIA* INVASION? EVIDENCES FROM SIMULATIONS AND FIELD RELEASES

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Field trials have started recently in Brazil to evaluate the spread of *Aedes aegypti* with Wolbachia, a bacteria that reduces arboviruses transmission such as dengue and zika viruses. Previous data reported stable and rapid invasion in Austrália and Vietnam using wMel strain. In Rio de Janeiro, we started weekly releases in Sep/2014, but Wolbachia frequency dropped dramatically soon after releases were suspended. Hindsight analysis showed that mosquito colony, which were closed after Australian *Ae. aegypti* females were backcrossed with Brazilian males, lost its alelles to pyrethroid (PI) resistance after only 14 generations in lab, suggesting a strong fitness cost due to insecticide resistance. Therefore, we released mosquito cohorts susceptible to PI in Rio which Ae. agypti populations face high insecticide resistance ratios for PI. Even releasing roughly 10,000 mosquitoes per week for 20 weeks, kdr frequency remained unalterd during releases. We hypothetized the insecticide susceptibility of released mosquitoes hindered Wolbachia invasion in Rio. We performed a new backcrossing with field males to produce wMelRio, a strain with similar insecticide resistance profile and fitness (survival and fecundity) when compared with field population. Thus, Wolbachia presence in a kdr mutated individual may exacerbate the fitness cost and could hinder Wolbachia invasion into a resistant population. In Jan/2016, after 24 weekly releases in field, wMelRio frequency was high as 80%. This frequency remains high (70-80%) even after a five-weeks period in which releases were stopped. Mathematical models were applied to test whether different releasing strategies (changing release number, wild population density, fitness cost of Wolbachia, fitness cost of insecticide resistance) would enhance Wolbachia invasion. Our simulations indicates a successful invasion in two situations: 1) releasing susceptible mosquitos in an environment without insecticide use (may causing a reversal in insecticide resistance levels); and releasing resistant mosquitoes (wMelRio) into a resistant population (such as Rio), even with a high insecticide use.

1850

WOLBACHIA INFECTION DOES NOT AFFECT THE DIVERSITY OF CO-INFECTING NATIVE FLAVIVIRUSES IN ADULT *AEDES AEGYPTI* IN THE FIELD

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Wolbachia (wMel) infections that have been artificially introduced into *Aedes aegypti* limit the ability of the mosquito to become infected with dengue and other flaviviruses in a trait known as pathogen blocking. It is unclear whether these antiviral effects would extend to other native flaviviruses in wild populations of mosquitoes. If so, Wolbachia may be beneficial in cases where these viral infections have negative effects on host fecundity and lifespan or induce energetic investments in immune responses. Here we examine whether the presence of Wolbachia infection in wild caught adults from field release populations in northern Australia reduces the native flavivirus diversity. RNA was extracted from adult mosquitoes collected from two sites within and one site outside of field release zones. Flavivirus specific primers for the NS5 region were then used to amplify from the converted cDNA of individual samples. Six insect flavivirus positive individuals were then selected from each of three sites for deep sequencing. We found that virus diversity was greater in the

Wolbachia infected mosquitoes, returning 10 different viruses versus 6 different viruses in the Wildtype samples. Cell fusion agent virus was found in all 18 samples across the sites and another 5 viruses were present at low levels in both wMel and Wildtype mosquitoes. A total of 6 viruses were unique to the wMel-infected mosquitoes. As the approach required PCR amplification prior to sequencing however it cannot address quantitative differences in the amount of viruses present. Future studies using non-targeted deep sequencing of insect material may address the issue around quantitation and also whether non-flaviviral diversity is impacted. Regardless, our study does not find clear evidence of Wolbachia's potential to benefit wild mosquito populations by limiting native flavivirus infections.

1851

LIGHT MANIPULATION OF MOSQUITO BEHAVIOR: ACUTE AND SUSTAINED PHOTIC SUPPRESSION OF BITING IN THE *ANOPHELES GAMBIAE* MALARIA MOSQUITO

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Host-seeking behaviors in anopheline mosquitoes are time-of-day specific, with a greater propensity of biting occurring during the dark phase of the LD cycle. We investigated how a short exposure to light presented during the night or late day can inhibit biting activity and modulate flight activity behavior. *Anopheles gambiae* s.s., maintained on a 12:12 LD cycle, were exposed to white light at the onset of night and the proportion taking a blood meal in a human biting assay was recorded every 2 hr for 8 hr. The pulse significantly reduced biting propensity in mosquitoes for up to 4 hr following administration, and with no differences detected after 6 hr. Conversely, biting levels were significantly elevated when mosquitoes were exposed to a dark treatment during the late day, suggesting that light suppresses biting behavior even during the late day. These data reveal a potent effect of a discrete light pulse on biting behavior that is both immediate and sustained. We expanded this approach to develop a method to reduce biting propensity throughout the night by exposing mosquitoes to a series of 10 minute pulses presented every 2 hr. We reveal both an immediate suppressive effect of light during the exposure period and 2 hr after the pulse. This response was found to be effective during most times of the night: However, differential responses that were time-of-day specific suggest an underlying circadian property of the mosquito physiology that results in an altered treatment efficacy. Finally we examined the immediate and sustained effects of light on mosquito flight activity behavior following exposure to a 30 minute pulse, and observed activity suppression during early night, and elevated activity during late night. As mosquitoes and malaria parasites are becoming increasingly resistant to insecticidal and drug treatments, there is a necessity for the development of innovative control strategies beyond ITNs. These data revealing the potent inhibitory effects of light exposure and the utility of multiple photic pulses presented at intervals during the night/late day, may prove to be an effective tool that complements established control methods.

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ESTIMATION OF ALLELE-SPECIFIC ACE-1 DUPLICATION IN INSECTICIDE-RESISTANT *ANOPHELES* MOSQUITOES FROM WEST AFRICA

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Variation in *Ace-1* copy number and G119S mutation genotype from samples of *Anopheles gambiae* across West Africa are used as appropriate strategies for identifying variation at population and individual levels. The most widespread and economical method, PCR-RFLP, suffers from an inability to discriminate true heterozygotes from heterozygotes with duplication. In addition to PCR-RFLP, in this study we used three different molecular techniques on the same mosquito specimens permitting comparisons. To group heterozygous individuals recorded from the PCR-RFLP analysis into different assumptive genotypes we used K-means clustering on the Z-scores of their correspondent data obtained from both TaqMan and ddPCR methods. Our data suggest that most heterozygotes are duplicated and that G119S mutation must now be regarded as a complex genotype ranging from primarily single-copy susceptible to Glycine and Serine allele balanced and imbalanced heterozygotes, and multiply-amplified resistant Serine allele homozygotes. Whilst qRTPCR-based gene copy analysis suffers from some imprecision, it clearly illustrates differences in copy number among genotype groups identified by TaqMan or ddPCR. Based on TaqMan method properties, and by coupling TaqMan and ddPCR methods simultaneously on the same type of mosquito specimens, we demonstrated that the TaqMan genotype assays associated with the K-means clustering algorithm could provide a useful semi-quantitative estimate method to investigate the level of allele-specific duplication in mosquito populations. *Ace-1* gene duplication is evidently far more complex in *Anopheles gambiae* than *Culex quinquefasciatus*, which consequently can no longer be considered an appropriate model for prediction of phenotypic consequences, which require urgent evaluation. Furthemore, if carbamates and organophosphate will be used as alternative products to pyrethroid for malaria vector control, the monitoring of duplicated alleles in natural populations of *An. gambiae* is essential to guide the rational use of these insecticides.

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INSECTICIDE RESISTANCE AND THE FUTURE OF MALARIA CONTROL

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The emerging and rapid spread of resistance to major classes of public health insecticides threatens current malaria vector control efforts namely long lasting insecticidal nets and indoor residual spray, which have contributed substantially to the reduction of malaria since 2000. The decreased ability of current vector control tools to effectively kill mosquitoes may be an early indicator to an increase in malaria cases and attributed deaths. Visualizing the confirmed reports of insecticide resistance in malaria endemic countries provides an indication where resistance may play a role in the persisting burden of malaria. Launched in 2012, IR Mapper is an online geospatial platform for mapping insecticide resistance in malaria vectors, built on a systematic review of peer-reviewed, published literature. The user interface enables filtering by country, year, vector species, insecticide class and type, and resistance mechanisms data including target site mutations and elevated metabolic mechanisms related to the detoxification of insecticides. As of March 2016, IR Mapper

consisted of 13,773 unique field records from 58 countries and 64 *Anopheles* species or species complexes. 78% of countries have reported resistance to at least one of the four classes of insecticides used for adult mosquito control. Examining the top ten countries with the largest burden of malaria today, more reports of confirmed pyrethroid resistance were recorded in the period 2008-2015 than compared to 2000-2007. Kenya and the Democratic Republic of Congo reported no pyrethroid resistance in 2000-2007 but in 2008-2015, 77.5 and 51.0% respectively of the testing conducted on pyrethroids reported resistance. In Burkina Faso, comparing the same time periods, the proportion of reports of confirmed pyrethroid resistance from the total number of tests conducted increased from 19.4 to 94.0%. IR Mapper is a useful tool for visualizing trends in *Anopheles* insecticide resistance and can be used to assist decision making for deployment of the most appropriate tools, which need to be driven by up-to-date data on insecticide resistance in target malaria vector species.

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THE EMERGENCE AND SPREAD OF INSECTICIDE RESISTANCE MUTATIONS IN *ANOPHELES GAMBIAE* AND *AN. COLUZZII*: INSIGHTS FROM DEEP WHOLE-GENOME SEQUENCING OF NATURAL POPULATIONS

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Insecticide resistance is a serious challenge to malaria elimination in Africa. We use haplotype data from the *Anopheles gambiae* 1000 genomes project to discover new mutations potentially linked with insecticide resistance, and to analyse the origin, distribution and movement of resistance mutations in populations spanning continental Africa. Within the voltage-gated sodium channel (Vgsc) we find kdr mutations in codon 1014 sweeping to high frequency in almost all Ag1000G phase 1 populations. We infer at least 4 independent origins for the L1014F kdr mutation and a further 4 origins for the L1014S kdr mutation. Some kdr haplotypes are found in a single population and thus have a local origin, whereas others are shared between populations separated by thousands of kilometers. One haplotype carrying L1014F has swept widely throughout West and Central Africa with at least two introgression events between species. Within Vgsc we also find 15 previously unknown non-synonymous mutations at high frequency. Of these, 13 occur exclusively on haplotype backgrounds carrying a mutation in codon 1014, suggesting selection for mutations that enhance or compensate for the resistance phenotype. We also find haplotypes sweeping to high frequency at three loci containing genes linked with metabolic resistance. At the glutathione S-transferase epsilon (gste) gene cluster at least four independent sweeps have occurred. One of the swept haplotypes carries the I114T mutation in gste2 known to enhance DDT metabolism and is found in populations from West, Central and East Africa indicating a continent-wide spread. The other high frequency haplotypes do not carry this mutation but do carry a large number of novel non-synonymous mutations which may be driving a resistance phenotype. We describe similar analyses for the cyp6 gene cluster and a locus on chromosome X containing cyp9k1. For all loci we identify SNPs tagging putatively resistant haplotypes as a basis for future monitoring efforts. These results reveal the threats posed by the capacity for mutations to spread throughout vector populations and to arise multiple times in different locations.

1855

HOUSEHOLD INTERVENTIONS, EXPENDITURES AND BARRIERS TO *AEDES AEGYPTI* CONTROL IN MACHALA, ECUADOR

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The *Aedes aegypti* is an efficient vector for the transmission of Zika, chikungunya and dengue viruses. However, understanding of the household expenditures needed to control this mosquito is relatively sparse. As various countries face the rising epidemic of *Ae. aegypti*-transmitted illnesses such as Zika virus, research on the extent of use and cost of interventions to control the *Ae. aegypti* is urgently needed. Between April to August 2015, we surveyed residents from 40 households in a high risk community in Machala, Ecuador on dengue knowledge and perceptions, vector control interventions, household expenditures, and barriers to employing prevention practices. Additionally, a semi-structured survey was recorded, transcribed and coded to identify the important factors that influence a household's decision to purchase mosquito control products. To determine the various types and cost of products available for sale to households, we surveyed 10 neighborhood stores and three modern supermarkets. The results of this study show that households in this neighborhood spend about 2% of their total family income on *Ae. aegypti* control interventions. On average, households concurrently employed five mosquito control interventions and had access to a variety of products, including aerosols, liquid sprays, repellents, mosquito coils, and unimpregnated bed nets. From our qualitative theme analysis, we found that effectiveness and cost were the most important factors that influence people's decisions to purchase a mosquito control product. These findings show a robust and healthy market for commercial mosquito control products even among the poorest of households in Machala, Ecuador. With the rise in Zika virus transmission, the need for *Ae. aegypti* control has only been exacerbated. Further research will examine how household economics are influenced by the rise of a new disease.

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RISK FACTORS FOR PEDIATRIC ENTERIC INFECTION IN A LOW-INCOME URBAN NEIGHBORHOOD: EXAMINING THE CONTRIBUTIONS OF THE HOUSEHOLD ENVIRONMENT, NEIGHBORHOOD GEOGRAPHY AND EXPOSURE BEHAVIORS IN VELLORE, INDIA

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Poor water, sanitation, and hygiene conditions contribute to pediatric enteric infection and longer-term health outcomes. In urban settings, child exposure to fecal contamination may be affected by the population density, physical characteristics of the neighborhood, and frequency of contact with fecal contamination both inside and outside the home. This study examined the contributions of a child's household and neighborhood environments and exposure behaviors to enteric infection risk, by etiologic agent, in an urban slum in India. Diarrheal and routine (monthly) stool were collected from 230 children during the first two years of life and assayed for enteric pathogens as part of the MAL-ED study. Exposures were assessed using spatial data and interviews with

caregivers in 100 of these households and evaluated using mixed effects logistic regression models. Household sanitation coverage (33%) and fecal sludge management (82% of household toilets discharged to open drains) were poor. Significant household risk factors, associated with 44-56% increased risk of any enteric infection, included the presence of, and open defecation by, older siblings and adult caregivers. Reported household-level water treatment (OR: 0.66, 95% CI: 0.50-0.88) and presence of a toilet (OR: 0.73, 95% CI: 0.55-0.97) were associated with reduced enteric infection risk. Residence in the spatial cluster of reported drain flooding, regardless of reported contact with drain or floodwater, was associated with significantly higher risk (OR: 2.39, 95% CI: 1.24-4.63) during the northeast monsoon. The risk factors associated with viral infections differed from those for any enteric infection, and included frequent use (>10 events/month) of public toilets as a unique risk factor for GII norovirus infection (OR: 2.05, 95% CI: 1.09 - 3.86). Overall, while some exposures, like the defecation practices of other household members, may be controllable within the household, conditions in the neighborhood environment may limit a household's ability to control health risks. Thus, interventions to reduce fecal contamination in the public domain are also necessary.

1857

ASSESSING SEROCONVERSION AGAINST ENTERIC PATHOGENS RELATIVE TO REPORTED DIARRHEA AND THE RECEIPT OF A POINT-OF-USE WATER FILTER IN WESTERN PROVINCE, RWANDA

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Diarrhea is a leading contributor to childhood morbidity and mortality in Sub-Saharan Africa. Given the infeasibility of blinding most water, sanitation and hygiene (WASH) interventions, diarrheal disease outcome measures in WASH intervention trials are fraught with potential bias and misclassification. We used the platform of a cluster-randomized controlled trial of a household-based drinking water filter in Western Province, Rwanda to examine the application of enteric seroconversion as an alternative and more objective outcome measure of current and recent infection. All children ≥ 6 and ≤12 months-old among 1582 study households were eligible for enrollment. All enrolled children had their blood drawn through capillary blood draw at baseline and 6 to 9 months after intervention distribution. Multiplex serologic assays for *Giardia lamblia*, *Cryptosporidium* parvum, Entamoeba histolytica, Salmonella spp., norovirus, *Campylobacter* spp., enterotoxigenic *E. coli* and *V. cholerae* were performed to compare seroconversion between the intervention and control groups on an intention-to-treat basis. The water filter was associated with a decrease in *Cryptosporidium* 17 kDa protein (Cp17) seroconversion (RR=0.72, 95%CI: 0.52-1.00) and serologic response against *Cryptosporidium* Cp17 was positively associated with reported diarrhea in the previous seven days (RR=1.78, 95%CI: 1.02-3.12). Children seroconverted against *Cryptosporidium* at relatively early ages (<6 months-old) while Giardia seroconversion typically occurred after 12 months. Serological responses for other antigens increased steadily after 6 months of age, plateauing after 12 months. Seroconversion shows promise as an objective outcome measure for WASH trials among children in this age group in addition to being a potential indicator of recent diarrheal disease.

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ASSESSING USE, EXPOSURE AND HEALTH IMPACTS OF AN ADVANCED WATER FILTER AND ADVANCED COOKSTOVE DISTRIBUTION PROGRAM IN RURAL RWANDA

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Unsafe water and air pollution are two major environmental health risks and contribute to child diarrhea and pneumonia. Household water filters and advanced cookstoves could reduce exposure risks, but there is little evidence of medium-term uptake and impact when combined. In 2012, a public-private program provided a free rocket stove and water filter to houses in 15 rural villages. We matched 9 intervention to 9 control villages using propensity score matching. Houses with a child under 5 (n=269) were enrolled and visited in 2 rounds over 12 months starting Nov 2013. At each visit, self-reported use, observed use, and reported health symptoms were recorded; a drinking water sample was also assessed for thermotolerant coliforms (TTC). Personal exposure to fine particulate matter ($PM_{2.5}$) was assessed gravimetrically in cooks (n=211) and children under 5 (n=172) for 48 hours. Use of the advanced filter and stove was assessed using sensors for 8 days each round. Overall, 92.7% of intervention houses had the filter, 90% reported currently using it, and 74.9% had water in it. Sensors indicated a daily average of 1.7L of water filtered/day (SD 2.5L). The control arm had a mean of 0.86 log TTC/100mL, compared to 0.37 log TTC/100mL in the intervention arm (p<.001). 95% of intervention houses had the stove, and 87% reported currently using it. Sensors indicated a daily average of 2.6 uses/day (SD 1.4 uses). Geometric mean (GM) $PM_{2.5}$ for intervention cooks was 156.1 μg/m^3 (95% CI 139.2-175.0) compared to 215.4 μg/m^3 for controls (95% CI 191.1-242.8); GM for intervention children was 171.2 μg/m^3 (95% CI 150.5-194.7) compared to 218.5 μg/m^3 for controls (95% CI 189.2-252.4). Among children in the intervention arm relative to control, mixed effects models showed a significant reduction in the odds of both caretaker-reported diarrhea (OR=.52, p=.04) and cough with difficulty breathing (OR=.11, p<0.001). We found high uptake and sustained use of a home water filter and advanced cookstove 12-24 months after intervention delivery, with evidence of reductions in drinking water contamination, household air pollution and improvements in reported child health symptoms.

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ENVIRONMENTAL EXPOSURE OF RURAL BANGLADESHI CHILDREN 3-18 MONTHS OLD FROM HAND- AND OBJECT-MOUTHING

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Children are exposed to environmental contaminants by placing contaminated hands or objects in their mouths. Exposure models based on mouthing data of children from high-income countries may be inappropriate to model exposure of children from different cultural and domestic settings due to differing mouthing frequencies. We sought to quantify hand and object mouthing frequencies of Bangladeshi children and determine if they differ from those of U.S. children, potentially indicating differences in types and levels of exposures. Trained observers used tablet computers to capture hand and object mouthing behaviors of 148 rural Bangladeshi children aged 3-18 months over five hours. For quality control, 11% of children were watched by more than one observer and inter-observer reliability was calculated. We analyzed the effect of

household and child characteristics on mouthing behavior and modeled mouthing frequencies using 2-parameter Weibull distributions to compare the modeled medians with those of U.S. children. Hand- and object-mouthing frequencies decreased with increasing age, and at all ages were higher than those of U.S. children. For hand mouthing, the median hourly frequency for Bangladeshi children 3-6 months old was 37.3 contacts vs. 23.0 contacts for U.S. children; for Bangladeshi children 6-12 months old, 34.4 contacts vs. U.S. 14.0 contacts; and for Bangladeshi children 12-18 months old, 29.7 contacts vs. U.S. 14.0 contacts. For object mouthing, the median hourly frequency for Bangladeshi children 3-6 months old was 23.1 contacts vs. 9.3 contacts for U.S. children; for Bangladeshi children 6-12 months old, 29.6 contacts vs. U.S. 19.0 contacts; and for Bangladeshi children 12-18 months old, 15.2 contacts vs. U.S. 12.3 contacts. Mouthing frequencies were not associated with child location (indoor/outdoor). Using hand- and object-mouthing exposure models from U.S. and other high-income countries might not accurately estimate children's exposure to environmental contaminants via mouthing in low- and middle-income countries.

1860

INCIDENCE OF ADULT DEATHS ASSOCIATED WITH HEPATITIS E VIRUS IN BANGLADESH

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Hepatitis E virus (HEV) causes acute infection of the liver. In addition to sporadic cases, HEV causes epidemic outbreaks in many countries in Asia and Africa, where fecal contamination of drinking water is common. A modelling study estimated 70,000 annual deaths globally from HEV. China has produced and licensed the first effective HEV vaccine but there are limited population-based data on burden of hepatitis E to take a rational decision of introducing HEV vaccine. In this study, we estimated the population-based incidence of adult deaths from HEV infection in Bangladesh. During Jan-Dec 2015, we conducted HEV surveillance in six tertiary hospitals in Bangladesh where we recruited all patients aged ≥15 years admitted with acute jaundice, defined as new onset of either yellow eyes or skin during the past 3 months. We collected blood from all cases to test for anti-HEV IgM using enzyme-linked immunosorbent assay. We conducted a mortality survey in the hospital catchment communites where we asked the caregivers of all deaths aged ≥15 years occurring in the community in the 3 years prior to the survey if the decedent had jaundice, defined as new onset of either yellow eyes or skin during the 3 months prior to death. We administered a verbal autopsy questionnaire for all identified jaundice associated deaths. We used a poisson model to estimate the incidence of jaundice associated mortality in the hospital catchment areas and then applied the proportion of laboratory confirmed HEV cases among patients admitted with jaundice to estimate the population-based incidence of adult deaths from HEV. We identified 519 patients admitted with acute jaundice; 441 of them were tested and 114 (26%) had laboratory confirmed HEV. In the hospital catchment communities with an adult population of 1,219,268, we identified 462 deaths associated with acute jaundice. The incidence of adult deaths associated with HEV was 3.6 (95% CI: 2.9-4.4) per 100,000 adult population per year. The study provides the first population-based estimate of adult mortality associated with HEV in Bangladesh, which can be used in economic evaluations of interventions, including HEV vaccine.

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WOMEN'S SANITATION EXPERIENCES ARE ASSOCIATED WITH MENTAL HEALTH IN RURAL, ODISHA INDIA

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Research on the impact of access to water and sanitation on health has focused on infectious agents and diseases, leaving other facets of health—like mental health (MH)—underexplored. No research has quantified how prevalent women's negative sanitation experiences are, how often they occur, and if their frequency and intensity influence MH. Qualitative studies suggest that women suffer assaults to MH due to poor sanitation conditions and negative experiences managing their needs, such as worrying about infection at urination and defecation sites, experiencing fear of potential harm, and limiting food and water to control urges. This study aimed to determine if a woman's negative experiences of sanitation—collectively designated here as sanitation insecurity— and her access to a facility were associated with anxiety, depression, distress, and well-being in rural Odisha India. We used an exploratory, sequential, mixed methods design to create a culturally-grounded measure of sanitation insecurity and test its association with MH. Our measure assessed seven domains of women's sanitation experience: Potential harms; Social expectations and repercussions; Physical exertion or strain; Night concerns; Dependent support; Physical agility; and Defecation location. From a survey with 1347 women, we found access to a latrine to be associated with higher well being scores, but not associated with anxiety, depression or distress. Women's sanitation insecurity domains were associated with all four MH outcomes, with most negatively associated with well being scores and positively associated with anxiety, depression, and distress scores. These relationships existed independent of latrine access. These findings imply that women suffer assaults to MH when urinating and defecating even if they own a facility. They suggest that sanitation interventions should accommodate women's experiences beyond management of excreta to more comprehensively impact health.

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THE IMPACT OF SANITATION INTERVENTIONS ON LATRINE COVERAGE AND USE: A SYSTEMATIC REVIEW AND META-ANALYSIS

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It is estimated that 2.4 billion people lack access to improved sanitation and 946 million practice open defecation. A further understanding of how different sanitation interventions impact latrine coverage and latrine use is essential in order to more effectively attain sanitation access for all. We systematically reviewed the literature and used meta-analysis to quantitatively characterize how different sanitation interventions impact latrine coverage and latrine use. We also used both qualitative and quantitative studies to assess how different structural and design characteristics of sanitation impact individual latrine use. A total of 59 studies met our eligibility criteria. We found 36 sanitation trials that, on average, found an increase in latrine coverage of 14% (95% CI: 10%-18%). The interventions with the largest increases in coverage included the Indian government's "Total Sanitation Campaign" (27%; 95% CI: 14%-39%), education interventions (17%; 95% CI 5%-30%), sewerage interventions (14%; 95% CI: 1%-28%), Community-Led Total Sanitation interventions (10%; 95% CI: 0%-20%), and other latrine subsidy/provision interventions that incorporated education components (17%; 95% CI: -5%-38%). Only 11 of these trials also assessed latrine use and in these the interventions had an average increase in latrine use of 13% (95% CI: 5%-21%). Individual study success was often context specific. Although many studies showed improvements in coverage and use compared to controls, these studies usually did not reach sufficient

coverage and use thresholds to translate into health impacts. We found 17 studies that examined how structural and design characteristics of sanitation were associated with latrine use. Better latrine maintenance, accessibility, privacy, facility type, cleanliness, newer latrine age, and better hygiene access were all frequently associated with higher latrine use, whereas poorer sanitation conditions were associated with lower use. A deeper understanding of how to effectively increase sanitation coverage and use could accelerate progress in eliminating open defecation and ultimately improve health.

1863

DISCOVERING AND OPTIMIZING BROAD-BASED ANTHELMINTICS USING PAN-PHYLUM ANALYSIS OF METABOLIC CHOKEPOINTS

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Parasitic nematodes infect more than two billion people, resulting in significant morbidity and mortality. The development of new therapeutics is indispensable due to the limited number of currently available drugs, their limited efficacy against some species (or life-cycle stages) and increasing anti-drug resistance. We undertook a systems biology approach to reconstruct metabolic pathways and to identify, characterize and prioritize chokepoint reactions and enzymes (that produce a unique product or consume a unique substrate) that we could target with test compounds to evaluate their potential for developing selective inhibitors against them. This is facilitated by recent extensive sequencing and annotation of genomes and transcriptomes of parasites spanning the phylum Nematoda, along with the availability of binding, structure, pharmacology and toxicology data for a large set of small molecules in publicly available databases. Preliminary work on a broadly conserved chokepoint enzyme CPT (Carnitine palmitoyl transferase) resulted in compiling of a small library of 12 CPT inhibitors (from DrugBank and ChEMBL databases or synthesized by us) that we screened against five parasitic nematode species spanning the phylum Nematoda. A worm motility assay identified 8 effective compounds, including 3 with potential for broad applicability across clades. An extension of this work to identify more such chokepoints is currently underway using enzyme annotation and metabolic reconstruction of 17 species spanning the phylum. A comparison of these resulted in identification of 202 chokepoints conserved across all taxonomic clades, including 87 that are conserved across all 17 species. These are currently being analyzed in order to prioritize a small number using multiple factors including level of conservation in nematodes and host, orthology in drug target databases, RNAi phenotype, expression profile across tissue and developmental stages, function in multiple pathways of interest, gene copy number etc. The chokepoints will be linked to inhibitors, and the predictions will be validated in multiple intestinal and filarial species.

1864

TRANSGENESIS IN *STRONGYLOIDES*: FREE-LIVING MALE WORMS AS TARGETS FOR GENE TRANSFER AND TRANSGENE PROPAGATION

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The capacity of parasitic nematodes in the genus *Strongyloides* to under go a generation of free-living development has enabled transgenesis in these worms by DNA transfer into oocyte nuclei. Anatomical similarity of free-living *Strongyloides* females to hermaphrodites of the free-living nematode *Caenorhabditis elegans* has made it easy to adapt DNA transformation techniques developed for this model organism to *S. stercoralis* and *S. ratti*. The technique involves microinjecting DNA constructs into the ovarian syncytium of free-living female *Strongyloides* and mating these with wild type male worms. This process yields only small numbers of F1 transgenic progeny (<100), a large proportion of which are somatic transformants only. In light of this, we examined free-living male *Strongyloides* as targets of gene transfer, reasoning that transgenic males could propagate transgenes to multiple progeny by mating and that the majority of transformed progeny would carry transgenes in their germlines. To this end we microinjected testes of 10 free-living male *S. stercoralis* with a solution containing 100 ng/µl of a proven reporter construct linking the coding sequence of green fluorescent protein (*gfp*) to the promoter of the ubiquitously expressed gene *Ss-eft-3* and the *Ss-era-1* 3'UTR. Microinjected males were plated along with 20 wild type free-living females on NGM agar with a lawn of *E. coli* OP50 and incubated at 22° C for mating. F1 larvae were screened for GFP expression at 24 and 48 hours in culture. Of 150 progeny screened, 3 (2%) were transgenic as indicated by GFP fluorescence in the anatomical patterns typical of the *Ss-eft-3* promoter. This indicates that the male germline of *S. stercoralis* may be transformed by microinjection of plasmid DNA and that transgenes may then be propagated to F1 progeny by mating with one or more wild type free-living females.

1865

MODULATION OF HUMAN DENDRITIC CELL ACTIVITY BY THE HELMINTH PARASITE *ASCARIS SUUM*

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Ascariasis currently affects more than 1 billion people worldwide. Like many other helminths, *Ascaris* is thought to actively modulate and/or down-regulate host immune responses and inflammation. The mechanisms behind this immune-regulation have not been fully elucidated, although modulation of dendritic cell (DC) and macrophage function is likely to be involved. Here, we investigated *in vitro* modulatory effects of *Ascaris suum* (a swine parasite closely related to the human *A. lumbricoides*) on human DC function. Monocyte-derived DCs were matured with the TLR-agonist lipopolysaccharide (LPS) in either the presence or absence of *A. suum* body fluid (ABF). DC function was assessed by analysis of cytokine secretion combined with transcriptomic and gene-set enrichment analysis (GSEA). ABF profoundly impacted on the response of DC to LPS. Secretion of the inflammatory cytokines IL-6, IL-12p70, IL-23 and TNF-α was strongly suppressed in ABF-treated DCs. Microarray analysis of ABF-treated DCs indicated a down-regulation of numerous genes encoding cytokines and chemokines, as well as molecules involved in intracellular inflammatory pathways and DC adhesion and migration. Selected genes were verified by qPCR and/or Western blotting. GSEA indicated significant disruption by ABF of numerous pathways involved in inflammation and DC maturation. Thus, we have demonstrated that *Ascaris* parasites strongly supress human DC function, suggesting that the parasite likely exerts a strong modulatory effect on the development of host immunity. These results increase our

understanding the host-parasite relationship in *Ascaris* infections and may contribute to the design of effective vaccines and other interventions for control of Ascariasis.

1866

GUT MICROBIOME CHANGES INDUCED BY EXPERIMENTAL *TRICHURIS MURIS* INFECTION ARE ASSOCIATED WITH DECREASED COGNITIVE FUNCTION IN MICE

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The effect of helminth infections on cognitive function of children has recently been under increased scrutiny. The gastrointestinal microbiome can modulate the functional development of the central nervous system. Recently we have demonstrated that helminth infections are able to induce important changes in the gastrointestinal microbiome of children. However, to date no studies have sought to evaluate the effect that microbial shifts associated with helminth infections may have on the host's cognition. This study aimed to investigate whether gastrointestinal helminthic infections are associated with decreased cognitive function as a result of changes to the gut microbiome. A chronic infection model was set-up using three groups of mice: two groups of 12 animals infected with *Trichuris muris* (a low and a high infection group) and 12 non-infected animals (control group). Mice were followed for 9 months and faecal samples were collected and stored in dichromate. Total DNA was extracted from the collected samples and changes in the structure and diversity of the gastrointestinal microbiome of mice in each group were done by evaluation of next-generation 16S rDNA sequencing. Cognitive function of mice was tested using the forced swim test (to identify depression-like endophenotype), working memory test (to measure general activity), a social interaction test and the reference Y-maze test (working memory). Our results indicate significant differences in diversity and abundance in the gut microbiome of mice in the control group compared to the low and high infection groups. Our results also indicated that mice in the high infection group show a deficit in reference memory compared to control and low infection groups associated with those alterations. This study demonstrates an alternative mechanism through which helminth infections can result in deficits in cognitive function. The functional profile of the groups of bacteria found altered and the clinical repercussions on cognitive delays of these alterations deserve further empirical studies in populations where both helminth infections and cognitive delays are highly prevalent.

1867

ASSESSING THE IMPACT OF MASS DEWORMING ON CO-INFECTIONS WITH OTHER PARASITES AND COMMENSALS USING MOLECULAR TECHNIQUES

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Studies in mice suggest that the presence of soil-transmitted helminths (STH) is associated with alterations in microbial community diversity. Some of these effects extend to anatomic locations within the gastrointestinal tract remote from where these helminths reside, and may persist after helminth clearance. Studies in humans have mostly failed to corroborate these findings. To demonstrate how molecular approaches to the human gut pathobiome and bacterial microbiome can provide insights into the complex interplay among disparate organisms, DNA was extracted from cryopreserved stools from subjects in 5 rural Kenyan villages and examined by qPCR for 9 intestinal parasites and by MiSeq 16S rRNA sequencing for bacterial communities before and 3 months following albendazole (ALB) therapy. Among 796 people surveyed by qPCR, 23% (186) had 2 or more gastrointestinal parasites concurrently. There were no strong inter-species relationships between the presence of one infection and the presence of any other parasite, except for an association between *Ascaris lumbricoides* and *Giardia lamblia* (Pearson chi-square, $p < 0.001$). Based on 16S rRNA sequence from 192 pre-ALB samples, there was no significant association between STH infection and microbial community composition. However, when a measure of microbial species diversity (Shannon index) was applied to 39 pairs of samples from individuals pre- and post-ALB, there was a significantly higher microbial diversity post ALB ($p=0.04$) in individuals who had *Necator americanus* infection pre-ALB, whereas there were no significant differences in microbial diversity pre and post-ALB in those with *A. lumbricoides* or those without any STH infection. We are currently sequencing additional samples, so that our final dataset will include pre- and post-ALB pathobiome and microbiome data from a much larger sample size (at least 60 pairs of samples for each of the important STHs and appropriate controls). This increased sample size will sharpen our understanding of the broader impact of mass deworming programs on microbial communities and ultimately on human health.

1868

CONTROLLED HUMAN HOOKWORM INFECTION MODEL FOR TESTING THE EFFICACY OF EXPERIMENTAL HOOKWORM VACCINES

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A controlled human hookworm infection model is being developed to provide early proof-of-concept that experimental hookworm vaccine candidates are feasible and efficacious. The proposed model consists of vaccinating healthy, hookworm-naïve adults with a candidate hookworm vaccine, followed by challenging them with infectious *Necator americanus* larvae (L3). We are conducting a feasibility study in Washington, DC, in which different doses of L3 are administered to healthy adult hookworm-naïve volunteers to determine the optimal dose that is safe, well-tolerated and results in consistent levels of infection. 3 cohorts of 10 healthy, hookworm-naive adult volunteers are receiving 25, 50, or 75 L3 in a dose-escalating design. L3 are obtained from the feces of an infected donor who is regularly screened for blood borne pathogens. Batches of L3 are tested for identity, motility/viability, and bacterial/fungal growth prior to release for use. Doses are prepared by counting motile L3 by microscopy; these are then applied to a gauze pad that is placed on the subject's forearm for 1 hour. Subjects are seen weekly until 12-18 weeks post-infection, when they are treated with albendazole. Results from the 25 and 50 L3 cohorts indicate that these doses are well tolerated by volunteers. Early manifestations of infection included mild-to-severe pruritus, erythema, pain, and papulovesicular rash (duration: 4-76 days) at the skin application site. Gastrointestinal complaints (abdominal bloating, flatulence, nausea and abdominal pain) were frequent starting between weeks 4-5 post-infection although these were mostly mild or moderate in intensity. Eosinophilia developed in 9/10 and 10/10 in the first and second cohorts, respectively (range: 0.5-4.9 x 10^3/mm^3). Hookworm eggs were detectable by microscopy in 3/10 (range by McMaster method: 0-33.3

eggs per gram [epg] feces) and 9/10 (range: 0-166.66 epg) in the first and second cohorts, respectively. Controlled hookworm infection with at least 50 L3 appears necessary to induce consistent infection in controls for future vaccination-challenge clinical trials.

1869

PHASE 1 TESTING OF THE NA-APR-1/ALHYDROGEL HOOKWORM VACCINE IN HEALTHY, HOOKWORM-NAIVE ADULTS

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Necator americanus Aspartic Protease-1 (*Na*-APR-1) is a 42.2-kDa protein produced by adult hookworms that is the first enzyme in the ordered cascade of hemoglobinases that the worms use to digest host hemoglobin. Vaccination of laboratory dogs and hamsters with recombinant APR-1 resulted in reduced hookworm fecal egg counts and reduced adult worm burden following challenge with infective larvae. Recombinant *Na*-APR-1 was derived from *Agrobacterium tumefaciens* genetically engineered to express the antigen in *Nicotiana benthamiana* tobacco plants and formulated on Alhydrogel. A Phase 1 trial was conducted in Washington, DC. 40 healthy, hookworm-naïve adults were vaccinated with 1 of 2 different dose concentrations of *Na*-APR-1 (30 or 100 µg) either with or without the point-of-injection addition of 2.5 or 5 µg of an aqueous formulation of glucopyranosyl lipid A [GLA-AF], a synthetic Toll-like receptor-4 agonist. Subjects received 3 intramuscular injections at 2-month intervals. In this study, the vaccine was well tolerated: common adverse events included mild to moderate injection site pain and tenderness, headache, and nausea. No differences were observed in adverse events between dose groups or GLA formulations. Mean anti-*Na*-APR-1 IgG antibody levels as measured by qualified indirect ELISA were modest after the 2nd vaccination, but increased significantly from baseline after the 3rd vaccination in those who received 100 µg *Na*-APR-1 (with or without GLA-AF). The highest peak IgG levels were observed in the cohort that received 100 µg *Na*-APR-1 in combination with 5 µg GLA-AF. In the 30 µg *Na*-APR-1 groups, mean IgG levels did not increase above baseline in those who received the Alhydrogel-only formulation whereas significant increases were observed after the 2nd and 3rd vaccinations in those who received *Na*-ARP-1/Alhydrogel plus GLA-AF. IgG responses were sustained until the end of the trial, 6 months post-final vaccination. This first-in-humans trial of the *Na*-APR-1 hookworm vaccine demonstrates that it is well-tolerated and immunogenic in unexposed healthy adults and justifies further clinical testing of this vaccine in endemic areas.

1870

IMPAIRED NEUTROPHIL RECRUITMENT TO INVADING *LITOMOSOIDES SIGMODONTIS* L3 LARVAE LEADS TO AN INCREASED WORM BURDEN IN NOD2 RECEPTOR AND IL-6 DEFICIENT MICE

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The NOD2 receptor is a widely spread intracellular pattern recognition receptor that is activated by muramyl-dipeptide (MDP), a bacterial cell wall component, triggering NFκB-induced pro-inflammatory responses. Since most human pathogenic filariae as well as the rodent filariae *Litomosoides sigmodontis* (L.s.) harbor endosymbiotic Wolbachia bacteria that synthesize the cell wall precursor lipid II, which contains MDP, we investigated the role of the NOD2 receptor during L.s. infection. Crude L.s. adult worm extract induced both NOD1 and NOD2 activation in NFkB reporter cell lines in a Wolbachia dependent manner. Upon infection with L.s., NOD2-/- mice harbored significantly more worms compared to wild-type (WT) controls. Lack of the NOD2 receptor did not change the cellular composition and analyzed cytokine/chemokine levels within the thoracic cavity, the site of worm residency. However, the skin stage of infection was essentially modulated in NOD2-/- mice, and bypassing the skin barrier by subcutaneous L3 injection resulted in a comparable worm burden in NOD2-/- and WT animals. Flow cytometric analyses and PCR arrays showed a significantly reduced neutrophil recruitment in the skin of NOD2-/- mice following intradermal injection of crude worm extract or L3 larvae, respectively. Further support that an impaired neutrophil recruitment mediates the increased worm burden in NOD2-/- mice was obtained by neutrophil depletion before natural L.s. infection, which significantly increased the worm recovery in WT, but did not alter the already elevated worm counts in NOD2-/- mice. That neutrophils are in general an essential part of the initial protective immune response against invading L3 larvae was further shown in IL-6-/- mice, which also had a delayed neutrophil recruitment within the skin resulting in an increased worm burden, which was not observed after subcutaneous infections. This study demonstrates that the NOD2 receptor is involved in protective immune responses against filarial nematodes by triggering the neutrophil-driven initial protective immune response against invading L3 larvae within the skin.

1871

MICROFILARIAE OF *BRUGIA MALAYI* INDUCES AUTOPHAGY THROUGH THE INDUCTION OF INDOLEAMINE 2,3-DIOXYGENASE (IDO) AND INTERFERON-γ (IFN-γ)

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Monocyte dysfunction in filarial infection is one of the mechanisms proposed to explain the diminished parasite antigen-specific T cell responses seen with patent filarial infection. In fact, monocytes from filariae-infected individuals demonstrate internalized filarial antigens and, as a consequence, express inhibitory surface molecules and have diminished cytokine production. To investigate the mechanisms underlying these phenotypic and functional changes induced by filarial antigens in monocytes we exposed purified human monocytes to live microfilariae (mf) of *Brugia malayi* and compared the mRNA and protein expression of important inhibitory immune checkpoint molecules to mf-unexposed monocytes. Our results indicate that mf significantly induced the mRNA expression of indolamine 2,3-dioxygenase (IDO) - a tryptophan catabolic enzyme with immune-inhibitory properties- in human monocytes and also significantly enhanced tryptophan degradation (an indicator of IDO activity; $p<0.005$) in these cells. As IDO induces autophagy through the upregulation and activation of GCN2 (a serine/threonine protein kinase), we next examined the expression of this kinase and autophagy related genes BCN1, LC3B, ATG5, and ATG7. Interestingly, mf significantly induced the mRNA expression of GCN2 and each of these autophagy related genes ($p<0.05$) in human monocytes. This upregulation was shown to be dependent on interferon-γ (IFN-γ) as mf significantly induced the production of this cytokine in monocytes ($p=0.03$) and a neutralizing anti-IFN-γ antibody reversed the expression of autophagy-related genes almost to the basal levels. Our data suggest that mf of *B. malayi* alter the function of monocytes by inducing IDO and IFN-γ, molecules that lead to monocyte autophagy that may in turn alter the host immune response.

1872

IMPACT OF MATERNAL PRAZIQUANTEL TREATMENT DURING PREGNANCY ON OFFSPRING IMMUNE RESPONSES TO SCHISTOSOME ANTIGENS AT SIX YEARS OF AGE IN LEYTE, PHILIPPINES: RESULTS FROM A RANDOMIZED CONTROLLED TRIAL

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Previous studies have suggested that schistosome antigens cross the placenta during pregnancy and influence newborn immune responses. We conducted a placebo-controlled trial of Praziquantel (PZQ) to schistosomiasis infected pregnant women in Leyte, Philippines. Here, we assess the impact of treatment on *in utero* sensitization to schistosome specific immune responses for N=107 six year old offspring of these mothers (55 PZQ, 52 placebo). We found no difference in schistosomiasis prevalence at age 6 (8.9% vs 13.5% in PZQ versus placebo, P=0.45). We purified PBMC from these children and stimulated them with schistosome worm (SWAP) and egg (SEA) antigens, and paramyosin. Cytokines (Interleukin-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, Interferon γ, TNF α) were measured in tissue culture supernatants using a multiplexed platform (Luminex). We evaluated differences in immune responses based on maternal treatment allocation using multivariate models adjusting for sex, schistosomiasis infection intensity and constitutive cytokine expression. Strikingly, children born to PZQ-treated mothers consistently demonstrated decreased levels (17% - 69%) of both Th1 and Th2 cytokines to SWAP (IL-12 1.33 vs 1.10 pg/ml (placebo vs PZQ), P=0.02; IL-13 53.7 vs 16.6 pg/ml, P=0.04, marginal for IL-4, IL-5, IL-13, IFNγ & TNF-α). We also detected a trend toward decreased levels of Th2 cytokines in response to SEA among children born to PZQ-treated mothers (IL-5 63.2 vs 23.9 pg/ml & IL-13 75.9 vs 29.4 pg/ml, both P=0.1). Interestingly, children born to PZQ-treated mothers had increased Th2 cytokine responses to paramyosin (IL-4 2.0 vs 2.3 pg/ml P=0.02; IL-5 3.2 vs 4.4 pg/ml, P=0.05), an immune response we have previously reported is associated with significant protection from infection. *In utero* sensitization was not associated with differences in IL-10 levels. Our data indicate that treatment modifies *in utero* immune sensitization to schistosome antigens, having potentially profound effects on parasite-specific cytokine profiles even at age six. We will present data from an additional 87 children by the time of the meeting.

1873

EFFECT OF PRENATAL EXPOSURE TO SCHISTOSOMIASIS AND CO-INFECTIONS WITH SCHISTOSOMIASIS ON FETAL IMMUNE RESPONSES

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Prenatal maternal infections are known to influence fetal immune responses. This fetal priming can enhance or suppress responses to parasitic antigens, which may influence the child's response to infection. Helminth infections are known to induce T-cell hyporesponsiveness and immunoregulatory mechanism. However, little is known on how helminth co-infections during pregnancy may impact on fetal immunity. We investigated immune responses from infants born to mothers with single or multiple infections. 79 Kenyan women were tested for the presence of *Schistosoma haematobium* (SH) and schistosome co-infection with filariasis and malaria (SH co-infected). Neonates' cord blood lymphocyte responses to SH worm (SWAP) and egg antigens (SEA) were analyzed for proliferation and production of IL-2, IL-5, IL-6 IL-10, IL-12, IL-13, IFN-γ, TNF-α and GM-CSF. Proliferation stimulation index (SI) and cytokine levels were compared between mothers with single (n=36), multiple (n=16) and no infection (n=27). Mothers with single infection at delivery were further classified based on treatment during pregnancy. Analysis was done using unpaired t test with welch's correction. Results showed a higher SI to SWAP, SEA and PHA (p=0.047) in the SH co-infected group (3.22, 2.77, 14.85) compared to the non-infected (1.26, 1.27, 4.96). Reduced IL-2, IL-5, IL-10, IL-12 and IFN- γ responses were recorded in SH and SH co-infected groups but levels did not differ significantly. In contrast, significantly lower levels of SEA stimulated GM-CSF (p=0.021) was recorded in the SH compared to the SH co-infected group. Mothers in the SH group who received anti-malarial drugs had higher levels of immune responses compared to the group not exposed to malaria. We noted spontaneous production of IL-6 in all groups. Conversely, significantly higher levels of spontaneous production of GM-CSF (p=0.041) and TNF-α was recorded in the SH co-infected group compared to the SH group. These preliminary results indicate that while schistosomiasis infection results in immunosuppression, fetal immune priming is enhanced with multiple infections and is sustained post treatment.

1874

LYMPHATIC FILARIASIS: HOST AND PARASITE FACTORS AND THE PATHOGENESIS OF SYSTEMIC ADVERSE EVENTS FOLLOWING TREATMENT

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Lymphatic filariasis (LF, aka "elephantiasis") is a neglected tropical disease (NTD) that is caused by the nematode parasite *Wuchereria bancrofti*. Some 800 million people in 73 countries are at risk for infection and disability caused by these parasites. Mild to moderate systemic adverse events (AEs) such as fever, myalgia, and headache are common after treatment of LF, and these AEs pose a major challenge for the global LF elimination program that is using mass drug administration (MDA) to interrupt transmission of the disease. We are studying the pathogenesis of AEs with blood samples collected before and after treatment in infected volunteers in clinical trials in Côte d'Ivoire and Papua New Guinea. We have used a Bio-Plex cytokine panel to measure 27 cytokines in 24 LF-infected individuals at seven time-points, from pre-treatment up until 72 hours post-treatment. Results show that 19 out of the 27 cytokines were significantly increased in post-treatment plasma in individuals with moderate AEs compared to individuals with no/or mild AEs. This included the three main pro-inflammatory cytokines (IL-6, TNF-α and IL-1β) that were all increased in people with moderate AEs between 8-36 hours post-treatment. Another interesting, and unexpected result was observed for Eotaxin-1. This eosinophil-specific chemokine was significantly up-regulated at baseline in individuals that would go on to develop moderate AEs after treatment. Eotaxin-1 could therefore be a potential biomarker for AEs risk. Preliminary results from global gene expression studies (RNAseq) suggest that several immune pathways are up-regulated in host leukocytes following treatment, and we hope to identify specific transcriptional signatures that are associated with AEs. Additionally, we have developed a qPCR assay for the detection of Wolbachia DNA in human plasma, and we have found that post-treatment plasma samples are more likely to test positive for Wolbachia. Improved understanding of the causes of post-treatment AEs may lead to improved methods for their prevention or management and increase compliance in mass drug administration programs that aim to eliminate LF.

1875

ONCHOCERCA VOLVULUS ANTIGEN PEPTIDE IMMUNOREACTIVITY DISTINGUISHES PARASITE POPULATIONS IN THE AMERICAS, WEST AFRICA, CENTRAL AFRICA AND EAST AFRICA

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Studies of *Onchocerca volvulus* (Ov) population biology may help elucidate its transmission, spread, emergence of drug resistance, and persistence despite control measures. Such studies are currently limited because they rely on the extraction of parasite material from their human hosts, material that is often limited in availability. Thus, we have developed a novel, serologically-based immunotyping approach to the study of *Ov* population diversity and spatial distribution. Using genomic sequence data and PCR-based genotyping, we identified non-synonymous single nucleotide polymorphisms (SNPs) in the coding sequences of many of the major known immunogenic *Ov* proteins: Ov 7, Ov 16, Ov ASP1, Ov CHI1, M3, Ov ALT1, Ov TMY1, Ov B8, Ov FAR1, Ov SOD1, Ov CPI1, Ov B20, Ov RAL1 and Ov RAL2. Using immunoassays to assess the antibody reactivity against synthetic SNP-containing peptides derived from these immunogenic proteins and well-characterized sera from a large cohort of patients (n=114) from multiple regions across Africa and the Americas, we have found statistically different geolocation-specific immunophenotypes (by Chi-Square analyses) against variant peptides derived from M3, Ov RAL1, Ov RAL 2, Ov SOD1, Ov CPI1, Ov B20, Ov RAL2, Ov TMY1, Ov16 and OvCAL1. Hierarchical clustering analysis also identified immunotype differences by region. Specific patterns of immunoreactivity against variant peptides from Ov B20, Ov TMY1, Ov16 and OvCAL1 clearly distinguished East African samples from those originating from Central Africa, West Africa and the Americas. Our data show that differences in immunoreactivity to variant antigenic peptides may be used to characterize populations of *Ov*, thereby shedding light on features of *Ov* population biology that may have been inaccessible because of the reliance on archived parasite material of limited availability.

1876

HAPLOTYPES WITHIN NFKBIA PROMOTER ARE ASSOCIATED WITH SEVERE MALARIAL ANEMIA AND CIRCULATING IL-10 AND IP-10 LEVELS IN CHILDREN WITH *PLASMODIUM FALCIPARUM* MALARIA

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Pathogenesis of severe malarial anemia (SMA, Hb<5.0g/dL and any density parasitemia) in children has been described as a multifactorial process. Genetic susceptibility factors have been proposed as elements of this pathogenesis. Transcription factors are important in regulating cellular processes including immunity. The inhibitor of nuclear factor of kappa light enhancer in B-cells (IκBs) plays important roles in infectious and autoimmune diseases through their ability to regulate the production of soluble immune modulators such as cytokines whose imbalance have been shown to characterize SMA. Due to the important roles of NFκB in immunity, we hypothesized that genetic variations within the promoter region of its inhibitor, IκBs (NFKBIA) gene affect its production thereby the downstream modulators of immunity and hence influence *Plasmodium falciparum* malaria outcome. The association between NFKBIA (-826 G/A, rs2233406 and -310 G/A; rs2233409 and SMA in children (n=1,026, aged 6-36mos.) with *P. falciparum* malaria from Siaya County, western Kenya, a *P. falciparum* holoendemic transmission area was determined. NFKBIA genotypes were determined using Taqman® genotyping assay. Bivariate regression analysis controlling for confounders revealed that existence of AA haplotype (NFKBIA-826A/-310A) was associated with risk of SMA (OR 1.60, 95%CI 1.01-2.55, P=0.047) while the AG haplotype (NFKBIA-826A/-310G) was associated with protection from SMA (OR; 0.58, 95% CI; 0.34-0.98). To identify the downstream target mediators modulated by NFKBIA, we used 25 mediators from Hu Cytokine 25-plex Ab Bead Kit. Additional analysis revealed that the AG haplotype (NFKBIA-826A/-310G) was associated with elevated levels of IL-10 and IP-10 (P=0.0050 and P=0.008, respectively). Moreover, SMA was associated with low levels of IL-10 and IP-10 (P=0.048 and P=0.025). These results demonstrate that genetic variation in the regulatory region of NFKBIA are associated with susceptibility to SMA and influence changes in the levels of circulating IL-10 and IP-10 during *P. falciparum* infection.

1877

PLASMODIUM MTRAP IS ESSENTIAL FOR GAMETE EGRESS AND PARASITE TRANSMISSION TO MOSQUITOES

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Apicomplexan parasites use an actin-myosin motor to glide on substrates and actively invade host cells. The TRAP (thrombospondin-related anonymous protein) family of transmembrane proteins, which is conserved in the apicomplexan phylum, is involved in parasite motility and cell invasion by integrating actin in the parasite and ligands in the matrix/cell surfaces. In *Plasmodium*, the family member expressed in the merozoite stage, called merozoite-TRAP (MTRAP), is thought to act during merozoite invasion into erythrocytes in the mammalian host. We show here that MTRAP is dispensable for this step but essential in the mosquito vector for gamete egress from erythrocytes, an actin and myosin-dependent process, by allowing the disruption of the membrane of the gamete-containing vacuole. This indicates that the apicomplexan TRAP protein family mediates more than parasite motility and cell invasion, and that vacuolar membrane disruption may result, at least in part, of a motor- and TRAP-dependent process.

1878

PROFILING GENE EXPRESSION IN A PHASE II *PLASMODIUM VIVAX* IRRADIATED SPOROZOITE VACCINE TRIAL

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Malaria remains an important public health problem worldwide, with 13.8 million cases caused by *Plasmodium vivax*, a parasite species that predominates in South-East Asia and the American continent. Despite the epidemiological importance of this species, studies of the immune response and their potential for vaccine development are limited. The vaccine that is in the most advanced stage of development is the anti-*P. falciparum* RTS,S vaccine, which has been reported to provide partial immunity when tested on a population of newborns and young children in West Africa. Development of more protective vaccines requires a better understanding of the human immune response. Here we report

initial results of gene expression profiling of peripheral blood before and after seven rounds of immunization with radiation attenuated *P. vivax* sporozoites (RAS) in 20 volunteers, as well as after controlled challenge with live *P. vivax*. RNASeq was used to generate whole transcriptome profiles for three non-immunized controls, five protected Duffy Fy-, five protected volunteers immunized with RAS, and seven not protected volunteers. The most remarkable changes in gene expression were observed between baseline and post- challenge, with distinct signatures differentiating protected and susceptible individuals. Analysis of transcriptional modules shows that B-cell signaling is reduced while cell cycle regulation and interferon response are highly elevated in individuals not protected by RAS, whereas T-cell signaling and an inflammatory response are elevated in protected individuals. Furthermore, some differences in the profiles associated with protection as a result of Duffy negative status and RAS immunization were observed, while vaccination itself also modified aspects of B and T cell gene expression. Combined with immune cell profiling we expect the systems biology approach may suggest novel approaches to improving the efficacy of malaria vaccines.

1879

HIGH-THROUGHPUT GENOMIC SURVEILLANCE OF *PLASMODIUM* INFECTIONS IN INDIA

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High-throughput genomic sequencing technologies provide the resolution, quality and rapid turnover required for routine large-scale surveillance of *Plasmodium* - for example for the spread of drug resistance genes or determining the complexity of infection. Here, we describe the development of a high-throughput amplicon sequencing protocol for multiplexed sequencing of multiple *Plasmodium* genes for routine surveillance purposes. We are using this in our Genomics Core Facility at the National Institute Malaria Research in New Delhi, India as part of an NIH-funded International Center of Excellence for Malaria Research, for surveillance of *Plasmodium* samples collected during our epidemiology studies. The amplicon sequencing protocol has been designed for the bench-top Ion Torrent PGM platform and can be operated with minimal bioinformatics infrastructure making it ideal for use in endemic country settings. In one assay, deep sequencing of a panel of six *P. falciparum* genes including *k13, crt, dhfr, dhps, mdr1* and *mrp1*, in ~150 clinical isolates from three epidemiologically diverse sites in India revealed a number of known and novel single nucleotide polymorphisms (e.g., in *Pfk13*), which could be associated with antimalarial drug resistance. In a second assay, we have shortlisted a panel of five *Plasmodium vivax* genes, including *msp1, msp3, sera5, msp7* and *clag* identified as being highly polymorphic across 200 *P. vivax* genomes, to estimate the number of clones in ~150 *P. vivax* infections. Our studies are revealing the within-host diversity of these isolates by using SeekDeep haplotype frequency estimation to infer the number of parasite clones and their change in frequencies after drug treatment. Our next-generation amplicon sequencing method facilitates surveillance of antimalarial drug resistance and helps elucidate the role of complexity of infection in disease outcome.

1880

REVERSIBLE HOST CELL REMODELING UNDERPINS DEFORMABILITY CHANGES IN MALARIA PARASITE SEXUAL BLOOD STAGES

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Survival of the human malaria parasite *Plasmodium falciparum* in the circulation of the host relies on its ability to drastically alter its red blood cell (RBC) host cell. The sexual blood stage (gametocyte) of the human malaria parasite *P. falciparum* undergoes remarkable biophysical changes as it prepares for transmission to mosquitoes. Developing mid-stage gametocytes show low deformability and sequester in the bone marrow, avoiding clearance during passage through splenic sinuses. Mature gametocytes exhibit increased deformability and reappear in the peripheral circulation, allowing uptake by mosquitoes. Here we define the reversible changes in RBC membrane organization that underpin this biomechanical transformation. Using a combination of biophysical techniques such as ektocytometry, spleen mimic filtration assays along with super resolution microscopy and atomic force microscopy techniques we functionally assess the role that RBC membrane skeleton remodelling plays in this reversible shift in deformability. We show that the length of the spectrin cross-members and the membrane skeleton mesh size increases in the non-deformable early gametocyte. These changes are accompanied by relocation of actin from the RBC membrane to the Maurer's clefts. These changes are reversed in the late stage gametocyte allowing parasite survival within the host and disease transmission.

1881

SPATIAL HETEROGENEITY CAN UNDERMINE THE EFFECTIVENESS OF COUNTRY-LEVEL TEST AND TREAT POLICY FOR MALARIA: A CASE STUDY FROM BURKINA FASO USING RDT AND HEMOGLOBIN

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Considerable debate has arisen regarding the appropriateness of the test and treat malaria policy recommended by the World Health Organization (WHO). While presumptive treatment has important drawbacks, the usefulness of test and treat can vary considerably across space, depending on several factors such as baseline malaria prevalence and RDT performance characteristics. Using 2010 Demographic and Health Survey (DHS) data, we fitted generalized linear mixed effects models to hemoglobin measurements, rapid diagnostic test (RDT), and microscopy results on 6,510 children under 5 years of age from Burkina Faso. Our statistical models revealed substantial variation in RDT performance, baseline prevalence, and hemoglobin measurements, both in space and as a function of covariates. As a result, an individual with a positive RDT result in one region can surprisingly have the same malaria infection probability as another individual with a negative RDT result in another region. These findings reveal that a test and treat policy might be reasonable in some settings but might be unacceptable in others given the high proportion of false negatives. Our results also suggest that in some regions RDT negative children that are severely anemic should be treated anyway for malaria. To aid the formulation of region-specific guidelines for malaria diagnosis

and treatment, we created proof-of-concept web-based tools for decision makers that enables them to interact with our modeling results. Our methods and results are likely to help improve current malaria policies in Burkina Faso and other malaria endemic countries.

1882

IMPROVING THE QUALITY OF MALARIA CASE MANAGEMENT IN PUBLIC HEALTH FACILITIES - THE MALARIACARE EXPERIENCE IN WESTERN KENYA

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Quality malaria case management remains a challenge in Kenya. To date, most health workers only receive occasional updates and irregular supervision visits which focus on infrastructural needs, not on skills assessment and improvement. In 2015, to address the deficits, MalariaCare is implementing a case management quality assurance (QA) program in eight counties around Lake Victoria. The QA strategy focuses on training QA teams of clinicians and laboratory technicians in the principles of quality malaria case management and mentoring and then supporting them in quarterly outreach training and support supervision (OTSS) visits using a structured checklist mentoring tool. After each OTSS round, county supervisors and health management teams will review data collected and design short-term approaches to address gaps at the county-level. To complete the QA cycle, following the first few rounds of OTSS, supervisors from multiple intervention counties will meet, along with national-level representatives, in lessons learned workshops, to exchange lessons learned across counties and develop action plans to address identified weaknesses. In addition, the project is working with hospitals to establish Medicines and Therapeutics Committees (MTCs) to assure that each case is managed according to quality assured protocols. The majority of public health facilities within the eight counties - including 71 hospitals, 185 health centers and 584 dispensaries - are being enrolled in three phases over 18 months. Using specific selection criteria, 75 laboratory technicians are receiving microscopy refresher training, 75 clinicians are receiving case management refresher training, and all are receiving OTSS supervision training. This report will describe the outcomes of these trainings and three rounds of on-site OTSS. The key indicators for microscopy, RDT, and clinical care performance will be discussed. The lessons learned from large-scale roll-out implementation of an electronic tablet-based and DHIS2-linked data collection system will be discussed, and the initial findings for implementation of the MTC system will be shared.

1883

FINDINGS FROM THE FIRST MALARIA MOLECULAR EQA SCHEME LAUNCHED BY UK NEQAS (UNITED KINGDOM NATIONAL EXTERNAL QUALITY ASSESSMENT SERVICE) PARASITOLOGY

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The WHO policy brief on malaria diagnostics in low-transmission settings recommends use of Nucleic Acid Amplification (NAA), and an international External Quality Assurance (EQA) system to ensure that data obtained are reliable and comparable. UKNEQAS Parasitology has developed such an EQA and the outcome of the pre-pilot and pilot surveys plus development of the EQA will be presented in this talk. A pre-pilot followed by a pilot survey for malaria molecular diagnosis using freeze dried blood samples was run. For the surveys, two distributions each containing lyophilised blood specimens were dispatched to an overall of 60 participants in 24 countries. The pre-pilot blood specimens contained parasite densities ranging from 20parasites/μL to 1parasite/μL. The pilot blood specimens contained parasite densities ranging from 40parasites/μL to 1parasite/μL. Both the surveys contained samples from single infections of *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. Negatives were also sent. Participants were requested to report on the presence or absence of malarial nucleic acid in reconstituted lyophilised blood using either qualitative or quantitative methods. The results showed reporting of higher false negatives at lower parasite densities whilst using both real time and nested PCR. Although a range of CT values and parasitaemia were reported by participants for each specimen, in general within a given assay the CT values increased with a decrease in the parasitaemia of the intended results, suggesting their ability to produce semi-quantitative results. In conclusion, the pre-pilot and pilot surveys demonstrate that NAA EQA schemes can successfully be run using lyophilised blood. Secondly, the majority of participants' results were in good agreement with the intended results. Thirdly, it fulfilled the EQA criteria in that the specimens might help participants take individual action to investigate and remedy any discrepant results. Fourthly, lyophilised blood usage obviates the need for a cold chain distribution, significantly reducing associated costs and opening up the distribution of such samples to a global audience.

1884

BUILDING A SYSTEM OF QUALITY ASSURED MALARIA CASE MANAGEMENT IN THE DEMOCRATIC REPUBLIC OF THE CONGO

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In the Democratic Republic of the Congo (DRC), assuring that microscopy and malaria rapid diagnostic tests (RDTs) are done properly and that test results are used appropriately remains a difficult challenge. To address this, the MalariaCare partnership has supported the National Malaria Control Program (NMCP) to implement a system of quality assured (QA) malaria case management. This system is designed on continuous improvement principles and includes updated training, certification of trainers, and on-site supportive supervision linked with regular review and feedback by supervisors. Over the past four years, 97 diagnostics experts have been trained in microscopy skills and RDT performance, and 17 clinical experts have been trained in malaria treatment. Best performers are then trained as onsite laboratory and clinical outreach training and support supervision (OTSS) supervisors and perform joint supervision and mentoring for local laboratory and clinical staff in 13 of the DRC's 26 provinces, including Kinshasa. The OTSS visits focus on skills observation and on-the-spot problem-solving, with primary goals of improving preparation and accuracy of malaria slide reading, assuring appropriate RDT results, and improving adherence by clinicians to test results. Between OTSS rounds 6 (June 2015) and 7 (February 2016), microscopy performance improved by 18 points—from 67% to 85% overall—and RDT performance improved by 50 percentage points—from 42% to 92% compliance with the performance checklist. Supervisors also observed an increase in clinicians correctly ordering a malaria test, from 64% to 90%, and correct prescription per diagnosis, from 8% to 52%. To assure steady quality improvement in the program, the supervisors meet to review outcomes and share best practices during annual lessons learned workshops. Based on these program improvements, the NMCP recently adopted this QA system as one of the key components in its new national strategic plan and will expand use of the system outside PMI supported health zones.

1885

SHIFTING THE PARADIGM: WHAT CAN BE DONE TO PROTECT COMMUNITIES AGAINST THE THREAT OF SUBSTANDARD AND FALSIFIED MALARIA MEDICINES?

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Substandard, spurious, falsified, falsely-labeled and counterfeit (SSFFC) malaria medicines pose an incredible threat to malaria endemic countries, as they not only put the individual at risk of treatment failure and death, but also increase artemisinin resistance, waste national healthcare system's limited financial resources and create distrust in the healthcare system. Unfortunately, these harmful medicines are quite common in low-income, malaria endemic countries, with recent quality assurance research finding that approximately one in ten doses of artemisinin-combination therapy (ACT) in sub-Sarahan Africa is poor quality. Most interventions designed to combat SSFFC malaria medicines work to improve quality assurance, address regulation policy reform, or operate within the criminal justice system, but few provide strategies to influence the demand for and purchasing practices regarding quality medicines. To this end, the Health Communication Capacity Collaborative (HC3) has developed a global initiative to unite stakeholders from regulatory agencies, criminal investigation units, clinical and pharmaceutical industries, national policymakers and program managers around promoting positive behaviors around malaria medicine purchasing, use, and reporting. HC3 will present findings from their pilot project in Akwa Ibom Nigeria, as well as introduce a step-by-step toolkit (Implementation Kit, or I-Kit) that can be used by any national or local entity to design and launch an effective program to combat substandard and falsified malaria medicines in their country or community.

1886

ANALYSIS OF ULTRA LOW COST NEAR-INFRARED SPECTROMETERS FOR DRUG AND BED NET QUALITY MONITORING

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The prevalence of falsified and substandard drugs is a barrier to effective management of malaria and other disease in resource constrained settings. Beyond drugs, other important healthcare interventions, such as long-lasting insecticide-treated bed nets. A simple low-cost tool to identify falsified and degraded commodities would secure supply chains, inform planning for product replacement, greatly reducing avoidable mortality. Handheld near-infrared spectroscopy (NIRS) systems have recently been developed for consumer use capable of distinguishing chemical composition of various materials, with hardware costs of $250 - 500. Spectroscopy is an attractive solution to drug and net quality testing due to low cost-per-test and the non-destructive nature of testing. The portability of these systems offer utility throughout supply chains. We investigated the capabilities of two portable NIRS devices, the Consumer Physics SCiO and the Texas Instrument NIRscan Nano, to perform quality assurance testing on a variety of drugs and nets. Spectral libraries were built for several classes of drugs including contraceptives, artemesinin combination therapies (ACTs), antibiotics, and others. The performance of these libraries was then tested in the laboratory and in field conditions with target users. Results were compared to reference testing with established reference standards, and with a laboratory-grade NIRS. Results include analysis of performance in falsified drug identification, active ingredient quantification (for finding substandard drugs), and active ingredient determination (for identifying unmarked pills). A bed net spectral library was compiled to quantify the presence of insecticide on the net. Both NIRS systems performed with high accuracy in identifying falsified drugs. In certain applications, active ingredients could be quantified sufficiently to assess degradation. Hand-held NIRS systems offer potential to revolutionize quality assurance of pharmaceuticals and other commodities in resource constrained settings.

1887

MALARIA INTERVENTION ASSESSMENT IN FOUR STATES OF NIGERIA: AN INNOVATIVE, COMPREHENSIVE, MIXED-METHODS EVALUATION

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The significant expansion of malaria control interventions in recent years has reduced the malaria disease burden in many countries, leading the global malaria community to set goals for elimination. In this context, there is need for appropriate tools and methods to document efforts and measure achievements. The Malaria Intervention Assessment (MIA), an innovative comprehensive evaluation methodology, funded by PMI, led by MEASURE Evaluation in partnership with the NMEP, is being implemented in four states of Nigeria supported by PMI: Cross River, Ebonyi, Nasarawa and Sokoto. The objective of MIA is to document progress in malaria control interventions from 2008-2016 in the four states. Specifically, MIA will describe the state-level malaria interventions; document trends in key malaria prevention and case management indicators and assess quality of care among PMI-supported and non-PMI-supported primary healthcare facilities (PHCs); document trends in malaria morbidity and mortality at the hospital level; assess the quality of monthly malaria data at PHCs; and document changes in contextual factors likely to affect malaria interventions and outcomes. MIA uses a quasi-experimental design and a comprehensive mixed-methods approach consisting of: (1) secondary data collation of malaria indicators from the routine health information system (RHIS) at 560 PHCs and their referral hospitals; (2) primary data collection, including: 2800 exit client interviews (5 at each PHC visited), 38 qualitative key informant interviews, and observations of the availability of malaria commodities at the PHCs; (3) secondary data analysis of state-level representative household surveys, and (4) document review. Using a stratified random sample with probability proportional to size, 140 facilities were selected in each state, 70 PMI-supported and 70 non-PMI-supported. Fieldwork will be completed in May 2016 so MIA results will be available to present at the ASTMH conference. The presentation will include key findings, strengths and challenges of MIA, and lessons learned for improving malaria control interventions and the RHIS.

1888

RAPID ACTIVE SEROPREVALENCE SURVEYS AS A TOOL TO MEASURE NOROVIRUS DISEASE BURDEN IN RESOURCE-LIMITED SETTINGS

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Cost-effective surveillance systems capable of accurately detecting acute gastroenteritis (AGE) are necessary to estimate the burden of pathogens such as norovirus (NoV) and the potential effectiveness of vaccines. Cross-sectional seroprevalence surveys are commonly used for outbreak investigations, but they have not been validated as a timely, community-based alternative to active surveillance. We used a 2-stage cluster design (30 clusters of 7 households) to enroll children age 0-17 years in a rural, resource-limited region of Guatemala into two parallel surveillance systems to estimate the burden of NoV-associated AGE. In the prospective Participatory Syndromic Surveillance (PSS) arm, 207 households with 483 children (enrolled Apr-Sep 2015) were provided an internet-connected smartphone with a symptom diary application and asked to submit weekly reports of AGE symptoms. Subjects meeting case criteria of 3+ days of vomiting/diarrhea or 1+ day of both were visited and offered NoV PCR testing via rectal or fresh stool swabs. In the Rapid Active cross-sectional Surveys (RAS), 377 children from 209 households (cycle 1), and 369 children from 210 households (cycle 2) from the same community were surveyed for AGE within the past 7 days and tested for NoV via PCR, regardless of symptoms. In the PSS arm, 50 children met AGE criteria during 362 person-years of observation (13.8 cases/100 person-years), and 9 of 34 (26%) tested were NoV+. In RAS cycles 1 (Oct-Nov 2015) and 2 (Jan-Feb 2016), 53 (14%) and 29 (8%) children had AGE in the preceding week and 6/39 (15%) and 5/24 (21%) tested were NoV-positive, respectively; the asymptomatic:symptomatic NoV ratio was 3.2:1; 79 (89%) of NoV isolates were genogroup I (GI) and 10 (11%) were GII. In logistic regression models adjusted for sex, younger age was a significant predictor of AGE but not NoV+ AGE. Our data demonstrate a large burden of NoV+ AGE and asymptomatic NoV shedding in this Guatemalan community. The more cost-effective RAS cross-sectional surveys provided comparable AGE incidence and NoV infection rates to the smartphone-based PSS active surveillance cohort, and further surveillance is planned.

1889

HIGH HEPATITIS E SEROPREVALENCE AMONG DISPLACED PERSONS IN SOUTH SUDAN: EVIDENCE OF UNDETECTED TRANSMISSION AND IMPLICATIONS FOR VACCINATION

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Hepatitis E (HEV) is responsible for significant morbidity and mortality worldwide, especially among pregnant women. Large protracted outbreaks have been documented in East African IDP and refugee camps over the past decade though little data on burden and transmission exist outside these exceptional settings. Characterizing the population-level exposure to the pathogen through age-stratified serological studies has the potential to improve our understanding of this disease and provide new insights for improving surveillance and control. We conducted an age-stratified serological survey among 206 residents in a camp for internally displaced persons in Juba, South Sudan where no clinical cases of HEV had been reported. We tested serum for HEV IgM and IgG using standard ELISAs and estimated the population-level prevalence of seroprevalence to each. Using data on individuals' sero-status, date of arrival in the camp and state of origin, we were able to construct a series of statistical models to estimate the rate of infection in the camp and that in the participants' previous residence. The age-adjusted seroprevalence was 61% (95% CI 54-69%) and we found evidence of recent exposure in 3 participants (1.5%). We found increasing IgG seroprevalence with age and higher seroprevalence in women compared to men. We estimate that the rate of HEV exposure was nearly 5-fold (95% CrI 1.2-10.2) higher in the PoC camp than our estimates in the participants' home states. The high seroprevalence estimated within this population suggests that HEV transmission may be much more common than previously thought, even in the absence of a detected outbreaks. The results suggest that the population is immunologically primed, which may have implications for control strategies, including vaccination, where a reduced-dose schedule may provide high levels of protection in immunologically primed individuals. Improved HEV surveillance is needed to understand the true burden of disease and to minimize the impact of epidemics.

1890

POSSIBLE HIGH EXPOSURE TO EBOLA AMONG NON-FORMAL HEALTH CARE PROVIDERS IN A PREVIOUS OUTBREAK SITE, BOENDE, DEMOCRATIC REPUBLIC OF CONGO

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During an Ebola virus disease (EVD) outbreak, health care workers (HCWs) are on the frontline of the response, and their occupational health and safety is critical to control and elimination of the outbreak. Thus they may be at increased risk of disease acquisition given the presence of virus in bodily fluids and lack of compliance with precautions to prevent exposure. Therefore, we conducted a serosurvey among formal and informal HCWs in the Boende health zone in Tshuapa District, DRC, the site of the 2014 Ebola outbreak. Field collection occurred in November 2015. Interviews and blood specimens were collected from all consenting individuals. Serum samples from 441 formal (doctors, nurses, midwives, and room attendants) and informal (religious leaders/pastors and traditional healers) HCW's were screened for ZEBOV GP Ig detection using Human Anti-Zaire Ebola Virus Glycoprotein (GP) IgG ELISA Assay kits (Alpha diagnostic International, Inc.) in Kinshasa, DRC. Among the HCWs, 21% (93) were seropositive for ZEBOV GP IgG, of those, 25% of pastors (n=27) were seropositive, 37% of traditional healers (n=27) were seropositive compared to 19.6% of formal HCWs. 27.6% of pastors reported that they had come in contact with suspected cases of Ebola, and of those, 87.5% reported that they did not use PPE compared to the 28.6% of formal HCWs reporting contact with suspected Ebola cases. Both formal and informal HCWs in Boende appear to be highly exposed to Ebola virus. While there appears to be no significant difference between formal and informal HCWs, the number of informal HCWs participating in the last outbreak was high. It is important that informal HCWs, especially in areas that have experienced EVD outbreaks, be included in surveillance and biosafety training in order to help prevent disease circulation in future outbreaks.

1891

SIERRA LEONE TRIAL TO INTRODUCE A VACCINE AGAINST EBOLA (STRIVE): IMPLEMENTATION CHALLENGES, SUCCESSES AND LESSONS LEARNED

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STRIVE, a phase 2/3 trial of investigational rVSV-ZEBOV vaccine was conducted during an unprecedented Ebola epidemic. Eligible health care workers and front line Ebola response workers were individually randomized to immediate (within 7 days) or deferred (within 18-24 weeks) vaccination and followed for 6 months for serious adverse events and Ebola. We describe lessons learned during trial implementation. Substantial infrastructure investments, including renovation of government cold chain facilities and importation of equipment to store and transport vaccine at -80oC, were needed. Generators and solar battery systems were used for backup. Staffing challenges centered on lack of experience with Investigational New Drug (IND) trials and their regulatory requirements. STRIVE built capacity by training >350 staff on IND research, including medical, pharmacy and nursing students whose classes had been cancelled during the outbreak. Didactic and practical training was reinforced with daily review and feedback meetings. CDC staff were paired long-term with local counterparts for role-specific skills transfer. The operational challenges of safety follow-up were addressed by issuing mobile phones to participants, establishing a nurse triage hotline, and providing access to free medical care. The effectiveness of these solutions was limited by frequent loss, breakage, or selling of study phones and frequent medical visits for minor ailments. Lessons learned include the need for back-up electrical and cold chain equipment, the importance of daily ongoing training supported by train-the-trainer approaches, the value of multiple participant locator information sources—including home visits—for participant follow-up, and the need for adequate staffing, systems, and guidance for free medical care. STRIVE enrolled ~8650 participants and vaccinated ~8,000 with excellent follow-up. Before the Ebola outbreak, Sierra Leone had limited infrastructure and staff to conduct clinical trials. Without interfering with the outbreak response, STRIVE responded to an urgent need and helped build this capacity.

1892

ASSESSING THE HETEROGENEITIES IN VIRAL HEMORRHAGIC FEVER OUTBREAK POTENTIAL ACROSS AFRICA

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As the Ebola virus disease outbreak in West Africa comes to an end, considering where to prioritise reinforcing response capacities to viral hemorrhagic fevers (VHFs) such as Ebola, Marburg, Lassa fever and Crimean-Congo hemorrhagic fever will be a key focus. By characterizing three key transition points in a potential VHF outbreak, this output provides the first quantitative assessment for districts in Africa which identifies those that are more likely to see index cases of VHFs, and should insufficient timely intervention measures be put in place, districts more likely to see localized outbreaks as well as those which are more likely to seed infection in other districts leading to a widespread outbreak. Information derived from zoonotic niche maps defining the geographic extent of the virus, coupled with measures of in-district healthcare infrastructural capacity and population vulnerabilities (inspired by a pre-existing index for risk management INFORM) as well as travel time surfaces are incorporated to provide this assessment across the African continent. The methodological framework is shown to be flexible to allow for improved, more disease specific covariates to be added as-and-when they become available. By understanding the inherent differences that exist across Africa, this method provides an alternative approach for identifying which districts to be targeted for broad scale healthcare improvement (focusing on VHF measures), as well as those to be prioritized for surveillance prior to outbreaks or the focus of rapid intervention should undiagnosed hemorrhagic fevers be reported.

1893

SPATIAL DETERMINANTS OF EBOLA VIRUS DISEASE RISK FOR THE WEST AFRICAN EPIDEMIC

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Although many studies have investigated the probability of Ebola virus disease (EVD) outbreaks while other studies have simulated the size and speed of EVD outbreaks, few have investigated the environmental and population-level predictors of Ebola transmission once an outbreak is underway. Identifying strong predictors of transmission could help guide and target limited public health resources during an EVD outbreak. A

Bayesian hierarchical Poisson model was used to estimate EVD risk and to evaluate the spatial variability explained by the selected predictors. We categorized our predictors into terciles, and found that districts had greater risk of EVD with increasing proportion of households not possessing a radio (RRRadio2 2.79, 0.90-8.78; RRRadio3 4.23, 1.16-15.93), increasing rainfall (RRRainfall2 2.18; 95% credible interval 0.66-7.20; RRRainfall3 5.34, 1.20-23.90), urban land cover (RRUrban2 4.87, 1.56-15.40; RRUrban3 5.74, 1.68-19.67), and years of education (RREducation3 1.58, 0.40-6.25). We found that districts with higher proportion of radio ownership had reduced EVD transmission risk, suggesting that that the use of radio messaging for control and prevention purposes may have been crucial in reducing the EVD transmission risk in certain districts, a potential modifiable risk factor for future outbreaks. Additionally, in areas with low proportion of radio ownership, public health authorities may need to develop and introduce different communication strategies. Future research should examine the etiologic relationships between the identified risk factors and human-to-human transmission of EVD with a focus on factors related to population mobility and healthcare accessibility, which are critical features of epidemic propagation and control.

1894

RE-CURRENT EPIZOOTICS OF HIGHLY PATHOGENIC AVIAN INFLUENZA IN NIGERIA AND STATUS OF VACCINATION AS ALTERNATE CONTROL

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Episodes of HPAI H5N1 in Nigeria are evidence of the risk of re-introduction of the virus through annual migration of infected waterfowls from Asia and Europe. Concerns are rife on the possibility of the virus becoming endemic in domestic poultry. This is moreso that the current wave of outbreak was not sufficiently contained leading to more cases of infection within six months than was ever experienced between 2006-2008. Current Federal Government status remains depopulation and decontamination without the use of vaccines. We however investigate evidence of vaccination by some poultry farmers desperate to prevention infection. In a limited prospective study in seven commercial poultry farms in South West Nigeria, 161 sera were randomly collected and tested by Agar Gel Immuno-diffusion (AGID) test to detect group specific nucleoprotein antigen of influenza A. Thereafter, Haemagglutinin Inhibition (HI) test was carried out using antigen directed against monospecific H5 subtype in a V-bottom microtitre plate with 1% solution of pooled chicken red blood cell as indicator. Eight (5%) sera had evidence of influenza A antibody shown by distinct precipitation line between antigen and antiserum in agar gel. Further analysis by HI showed three (2%) sera were positive for influenza antibody at low titer HI of 3log 2. This study for the first time, showed evidence of antibody to avian influenza in domestic flock in Nigeria that is most likely due to vaccination. Previous serological tests in farms infected with HPAI H5N1 were negative. There are unconfirmed reports of vaccination in southwestern region, the hub of poultry production in Nigeria. Unregulated and inappropriate application of vaccine may results in poor antibody responses as demonstrated in this study. In view of these possibilities, it is in the best interest of avian influenza disease control to monitor the use of vaccines in commercial poultry and immune responses thereof.

1895

USE OF A QUANTIFIABLE STOOL RT-PCR ASSAY INCREASES DIAGNOSTIC YIELD IN CHILDHOOD TB

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Quantification of Mycobacterium tuberculosis (Mtb) has potential to improve TB diagnostics and treatment monitoring, with particular relevance in childhood TB as its paucibacillary nature presents a diagnostic challenge. We evaluated the diagnostic yield of quantitative real-time PCR for the detection of Mtb in stool. Stool was collected from a cohort of adults and children with confirmed or clinically diagnosed TB (n=67). DNA was isolated from 50mg of stool using the MP Fast DNA soil kit. Tuberculosis-specific primers were designed from the IS6110 insertion element found exclusively within the Mtb complex. To determine a limit of detection (LOD), 10 to 106 CFU of H37Rv Mtb was spiked into 50mg of healthy stool and the DNA was isolated. All samples were analyzed using quantitative real-time PCR. The LOD of Mtb was < 10 CFU of Mtb per 50mg of stool. The CFU of Mtb spiked into stool and DNA quantified by PCR was well correlated (Figure 1A: Spearman r = 0.998, p< 0.0001). The quantity of Mtb DNA detected inversely correlated with time on ATT (Figure 1B; Spearman r = -0.4219; p = 0.04). The quantified Mtb DNA in stool at 2 months was lower than baseline levels (Figure 1C, Wilcox signed rank test; p< 0.0001). Stool qPCR had similar diagnostic accuracy as sputum GeneXpert Mtb/RIF (Xpert) amongst individuals who had stool collected within 72 hours of ATT initiation (Fisher´s exact p-value 0.32). Stool PCR identified 15% (4 of 26) children who were clinically diagnosed with TB despite having negative Xpert and culture results. In conclusion, detection of Mtb DNA from stool provides a quantifiable measure of an individual's Mtb burden. PCR detection of Mtb in stool of children with clinically diagnosed TB (Xpert and culture negative) highlights the potential for this assay to increase bacteriologic confirmation of childhood TB.

1896

IS IT TINDZHAKA OR TUBERCULOSIS? A STUDY OF TRADITIONAL DIAGNOSIS AND TREATMENT AMONG HEALERS IN BUSHBUCKRIDGE, SOUTH AFRICA

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The prevalence of Tuberculosis (TB) in South Africa (SA) has increased significantly over the last 30 years, with an annual incidence rate of 1% (roughly 450,000 new cases per annum). In Bushbuckridge, SA, Tindzhaka is a common condition for which people seek the services of a traditional healer. Tindzhaka is an ailment that affects the lungs and can eventually lead to death. There is concern among clinicians that Tindzhaka and TB are the same illness; increased understanding about the causes, symptoms, treatment, and expected outcomes associated with Tindzhaka can be used to engage healers to support testing of suspected TB patients. In 2015, we completed 27 in-depth interviews and 133 surveys with a simple random sample of traditional healers in rural Bushbuckridge, SA. Healers were mostly female (77%), older (median = 58 [IQR: 50-67 years]), with low levels of education (median = 3.7 [IQR: 3.2-4.2]) years). Seventy-three percent of healers claimed to treat Tindzhaka (while less than 10% claimed to treat TB). Our research has revealed the overlapping symptoms of Tindzhaka and TB, including coughing, difficulty breathing, loss of body weight, fevers and, ultimately - if there is no treatment - death. Healers argue these to be two distinct illnesses. Color of the sputum (white indicates Tindzhaka, while yellow indicates TB) was identified as one means of distinguishing the two illnesses. Several social transgressions are

believed to cause Tindzhaka infection: (1) having sex with one's partner before the family member's death ceremonies are completed; (2) having sex with one's partner too quickly after a funeral; or (3) bringing home any items (including food from the funeral) from the deceased member's house. On average, healers charged patients with Tindzhaka 1376 SA Rand (IQR: 600-1500; 82 United States Dollars (USD)) and patients with TB 700 SA Rand (IQR: 400-1000; 47 USD) for treatment. With 11% mortality among those who contract TB in SA, widespread acceptability of traditional treatments for an illness with similar presentation may contribute to poor patient outcomes. Further engagement with traditional healers is required.

1897

FOLLOW-UP EVALUATION IN THE UNITED STATES OF NEWLY ARRIVED IMMIGRANTS AND REFUGEES AT HIGH RISK FOR TUBERCULOSIS, 2009-2015

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Required culture-based overseas tuberculosis (TB) screening in U.S.-bound immigrants and refugees reduces importation of TB to the United States. It also identifies persons at high risk for TB, for whom post-arrival follow-up evaluation is recommended. We analyzed data collected by state and local health departments to determine TB rates among at-risk newly arrived immigrants and refugees. During 2009-2015, overseas screening identified 92,248 U.S.-bound immigrants and refugees with Class B1 Pulmonary TB (chest radiograph, medical history, or examination suggestive of TB but smear- and culture-negative) and 57,475 with Class B2 Latent TB Infection (LTBI). Of 62,131 persons with Class B1 Pulmonary TB who completed follow-up evaluation, 1,028 were diagnosed with active TB within 1 year after arrival; of 403 culture-confirmed cases, 3.0% (12) had multi-drug resistant TB, 0.7% (3) were resistant to isoniazid, 6.2% (25) resistant to rifampin, and 3.7% (15) resistant to other first-line drugs. Of 36,068 persons with Class B2 LTBI who completed follow-up evaluation, 121 were diagnosed with active TB; of 13 culture-confirmed cases, 7.7% (1) were resistant to first-line drugs other than isoniazid or rifampin. TB rates were 1,655 and 336 cases per 100,000 persons for those with Class B1 Pulmonary TB and Class B2 LTBI within 1 year after their arrival, respectively. For persons with Class B2 LTBI, TB rates were 3,268, 308, and 531 cases per 100,000 persons for those aged <2, 2-14, and ≥15 years, respectively. Of 98,199 persons with a Class B1 Pulmonary TB or Class B2 LTBI, 40,718 persons were diagnosed with LTBI by follow-up evaluation, 25,054 (61.5%) initiated preventive therapy but only 11,118 (27.3%) completed their treatment. Newly arrived immigrants and refugees have high rates of active TB, despite overseas screening. High TB rates among persons with Class B2 LTBI in all ages suggest that expanding overseas LTBI screening beyond the currently required 2-14 years should be considered. To further prevent TB in the United States, strategies are needed to improve the completion of follow-up evaluations for all persons, and preventive therapy for LTBI.

1898

USING POINT-OF-CARE C-REACTIVE PROTEIN TEST RESULTS TO TARGET ANTIBIOTIC PRESCRIPTION FOR RESPIRATORY ILLNESSES IN UNDER-FIVES: EXPERIENCE FROM A CLINICAL TRIAL IN DAR ES SALAAM, TANZANIA

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We sought to determine the usefulness and safety of using C-reactive protein (CRP) test results in deciding on antibiotic prescription for respiratory illnesses among febrile children presenting to outpatient care. A sub-cohort of all patients with cough and no signs of severe illness from a larger trial that investigates a novel electronic algorithm for management of fever among under-fives in Dar es Salaam, Tanzania, was included. A two-step diagnostic approach was used to decide on antibiotic prescription: amoxicillin was given if a patient presented with i) respiratory rate (RR) between the 75th and <97th %ile for age and temperature based on a European derivation study as well as ii) CRP ≥80mg/L using a point-of-care assay (Bionexia™, Biomerieux). All children were followed until clinical cure or death. Out of the 922 patients with cough, 428 patients met the 75th %ile cutoff for RR, of which 277 (64.7%), 115 (26.9%), 26 (6.1%), and 10 (1.1%) patients had CRP levels of 0-9, 10-39, 40-79, and ≥80mg/L, respectively. Antibiotics were thus prescribed in 10 (1.1%) of patients. Out of the 428 patients, 9 patients met clinical failure criteria per the main study at day (D)3 or D7 (7 had CRP values of 0-9, 2 of 10-39mg/L) : 2 developed severe respiratory symptoms, 3 had persistent fever at D7, 4 still had clinical pneumonia with low CRP values at D3 but recovered before D7 without antibiotic treatment, and 1 patient had clinical pneumonia at D7. There were no deaths. Using current IMCI cut-offs, 226 (53%) out of these 428 patients would have been prescribed an antibiotic at presentation. A two-step diagnostic approach using respiratory rate and a CRP ≥80mg/L is safe for deciding on antibiotic prescription among febrile children with respiratory symptoms and has the potential to significantly reduce antibiotic prescriptions. Further research should be conducted in children at higher risk for bacterial pneumonia, i.e. in areas with high rates of malnutrition and low immunization coverage. In addition, newer host biomarkers with better performance should be evaluated in clinical studies.

1899

WHAT DROVE THE DECLINE IN PNEUMONIA-SPECIFIC UNDER-FIVE DEATH IN MALAWI FROM 2000-2014?

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Globally, pneumonia is the leading cause of mortality in post-neonatal children under 5 years of age. More than 95% of the estimated 0.9 million under-five children who died from pneumonia in 2013 lived in low and middle income countries. Overall mortality in children under five years of age has declined dramatically in the past ten years in Malawi, partly due to decreases in childhood pneumonia. We explore how scale-up of specific interventions contributed to this decline. Our objective is to determine

which interventions contributed the most to reducing pneumonia-specific mortality in Malawi from 2000 to 2014. We used the Lives Saved Tool (LiST; Spectrum v5.41 Beta 6) to conduct a retrospective analysis to estimate change in pneumonia-specific under-five mortality over the study period. Estimates of intervention coverage were drawn from Malawi Demographic Health Surveys (MDHSs) from 2000, 2004, and 2010, and the Multiple Indicator Cluster Survey (MICS) from 2006 and 2014. Data were interpolated from existing data for years without coverage estimates. Key outcomes included reduction in under-five mortality due to pneumonia and lives saved by pneumonia-specific interventions. Preliminary results show that among children under five, pneumonia-specific mortality declined by 59% among children aged 1-59 months from 2000 to 2014. Although the number of neonatal deaths due to pneumonia has been decreasing since 2006, neonatal pneumonia deaths was slightly higher (8%) in 2014 relative to 2000. Nearly all (98%) of the lives saved were attributable to vaccination (37%), including H. influenzae b and pneumococcal conjugate vaccine, antibiotics for treatment of pneumonia (34%), and various interventions to reduce stunting and wasting (27%). Overall pneumonia-specific mortality in children under-five has declined sharply in Malawi since 2000. Treatment and prevention both played key roles in saving lives. Ongoing implementation of interventions is essential to maintain this trend.

1900

EPIDEMIOLOGY OF HUMAN METAPNEUMOVIRUS IN CHILDREN UNDER AGE FIVE — DAMANHOUR DISTRICT, EGYPT, 2009-2015

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In 2013,Acute Respiratory Infection (ARI)was the 5thleading cause of under-five mortality in Egypt.Human metapneumovirus (hMPV) was the second most common cause of ARI. However, data is only available through sentinel surveillance. We sought to estimate the incidence and describe the characteristics of hMPV among ARI cases from population-based surveillance in Damanhour, Egypt. During June 2009-December 2015, hospitalized ARI patients were enrolled from three government referral and two private hospitals. An ARI patient was defined as having a temperature ≥38°C or <35.5°C, abnormal white blood cell count or differential, at least one respiratory symptom, and age<5 years. Nasopharyngeal and oropharyngeal specimens were tested by real-time reverse transcriptase polymerase chain reaction (rt-PCR) for hMPV. Data from a 2012 healthcare utilization survey were used to determine the proportion of individuals who sought care for ARI. Frequencies and chi-square test were used for data analysis. Among 4,400ARI cases, 4181(95.0 %) had rt-PCR testing for hMPV; of these, 322 (7.7%) were positive. hMPV was the only pathogen in 276 (85.7%) cases. Overall, 255 (79.2%) were rural residents and 185 (57.5%) were male. The highest proportion of hMPV infections among ARI cases occurred during December-February (214/1503, 14.2 %) compared to other months (108/2897, 3.7%), (p<0.01).Overall incidence of hMPV infection was 2.3 per 1,000 child-years. hMPV infection increased over time (p<0.01) with the highest proportion occurring in 2010(12.5%).hMPV patients presented with sudden onset of fever (99.4%), cough (99.4%), abnormal breathing (82.9%),and tachypnea (55%).Among 197 patients with chest radiography, 43(21.8%) had consolidation. Mean duration of symptoms was 4.9+3.1days and hospitalization was 4.0+2.6 days. Thirteen (4.0%) patients were admitted to intensive care for a mean duration of 4.8 (+2.5) days. Two patients died, both under age two years. hMPV infection peaked during winter and is a significant cause of ARI in children under age five years in Damanhour District, Egypt.

1901

ETIOLOGY OF ACUTE LOWER RESPIRATORY INFECTIONS IN INPATIENT CHILDREN IN GHANA - A CASE-CONTROL STUDY

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Background: An estimated 14.5 million pediatric hospital admissions are caused by acute lower respiratory tract infections (ALRI) each year. Molecular diagnostic tools have led to the detection of a large number of organisms claimed to be causal in ALRI. However, current data are insufficient to determine whether these viruses are truly pathogenic. The aim of this study was to assess the prevalence, pathogenicity and clinical relevance of organisms in respiratory infections of Ghanaian children using a case-control design. Methods: From September 2014 to August 2015 all children admitted to a Hospital in Ghana with symptoms of ALRI were recruited. Healthy controls were recruited from the community. Pharyngeal swabs were analysed by PCR for Pneumococci, Mycoplasma, Influenza (A, B), Parainfluenza (1-4), hMPV, RSV, Entero-. Rhino-, Adeno-, Parechovirus and Human Corona Viruses (NL63, 229E, OC43, HKU-1). The frequency of organisms in both groups was determined and age-adjusted odds ratios (OR) for association with ALRI calculated using logistic regression models. The attributable risk fraction of ALRI for each organism was estimated. Results: 337 children were recruited as cases and 573 as healthy controls. Of these, 235 (69.7%) and 271 (47.3%) tested positive for at least one organism in the case and control group, respectively. The most common organisms in cases were pneumococci (163, 48.4%), Adenoviruses (60, 17.8%) and Rhinoviruses (59, 17.5%). In the control group, pneumococci (176, 30.7%), Rhinoviruses (145, 25.3%) and Enteroviruses (113, 19.7%) were the most frequent organisms. The strongest association with ALRI symptoms was seen for influenza viruses (OR=32.3; 95% CI 7.7-136.0; p<0.001) and RSV (OR=12.3; 95% CI 3.6-42.0; p<0.001). The highest attributable fractions were 28.4% for pneumococcal infection and 9.6% for influenza virus infection. Discussion: Strong associations of influenza, RSV and hMPV with disease, indicate that these are most likely causative if detected in a child with ALRI. Despite the introduction of a vaccine 2 years prior to the study, pneumococcal infection was still the most important cause of ALRI.

1902

COMPARISON OF THE HUMORAL RESPONSE INDUCED BY DIFFERENT LINEAGES OF *TRYPANOSOMA CRUZI* IN A MURINE MODEL

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Trypanosoma cruzi is classified based on genetic variability into six discrete typing units (DTU TcI to TcVI). This genetic diversity may be related with the clinical features and humoral immunity observed in Chagas´s disease. The aim of this work was to evaluate the humoral responses induced by two different strains of *T. cruzi* (DTUs TcI and TcV), from an endemic area of Argentina. We used female BALB/c mice 45 days old infected intraperitoneally with 10.000 metacyclic trypomastigote forms. For the

measurement of antibody titers induced by the parasites we used an in-house ELISA. Serum samples were taken at different time points, measured in days post-infection (dpi), since 8 to 120 dpi. As capture antigens we used protein homogenates (HP) of two different strains of *T. cruzi* (TcI, TcV). The homogenates were characterized by SDS-PAGE. We carried out two tests: ELISA-HPTcI and ELISA-HPTcV for each experimental group. Each serum set reacted with the antigens, demonstrating the presence of antibody anti-*T. cruzi* in the experimental groups. We observed a high sensitivity and specificity of the reaction between serum and antigen of the same DTU. The values of optical density (OD) in serums of mice infected with TcI were significantly higher ($p < 0.05$) than serum of animals infected with TcV, when HPTcI was used. However, when HPTcV was used, we observed the highest OD in mice infected with TcV. On the other hand, we observed difference in the kinetics of antibodies. Serum of TcI-mice presented an exponential increase in the antibody titers along time of the infection. While serum of TcV-mice showed the highest antibody titers at 90 dpi and then decreases. By SDS-PAGE technique we observed differences in the protein profile of each homogenate. In conclusion the results suggest that strains TcI and TcV induce different serological responses according to the antigen used. On the other hand the different proteins of each antigen could participate in the specificity and sensitivity of the technique used. These findings are potentially useful in the search for new antigens to be applied in serological tests or as molecular markers.

1903

PHENOTYPIC AND FUNCTIONAL CHARACTERISTICS OF HLA-DR+ NEUTROPHILS IDENTIFIED IN CIRCULATION OF BRAZILIAN CUTANEOUS LEISHMANIASIS PATIENTS

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The vector-borne protozoan *Leishmania braziliensis* causes the chronic ulcerative skin disease cutaneous leishmaniasis (CL) in individuals living in endemic regions. In murine models, neutrophils (PMNs) are recruited to the site of infection minutes after parasite inoculation, but their role during chronic infection, and the role of PMNs in human disease, remain undefined. We hypothesized that PMNs from patients with active CL would exhibit different functional properties compared to healthy subjects. Despite the fact that CL is a localized disease, a subset of CL patients had circulating neutrophils expressing HLA-DR, a molecule thought to be restricted to professional antigen presenting cells. We also examined lesion-recruited PMNs for these same surface markers. Circulating HLA-DR+ PMNs also expressed the co-stimulatory molecules CD80, CD86 and CD40. Recently described low-density PMNs contain a high percentage of HLA-DR+ PMNs. Sorted HLA-DR+ PMNs morphologically resembled conventional PMNs, and they were capable of phagocytosis and reactive oxidant generation. Nonetheless, PMNs from subjects with high proportions of HLA-DR+ PMNs promoted significant *in vitro* proliferation of T cells. Compared to conventional HLA-DR- PMNs, HLA-DR+ PMNs showed increased activation, degranulation, oxidant generation and phagocytosis of parasites and zymosan particles. Incubation of whole blood with inflammatory cytokines resulted in increased HLA-DR+ PMNs, suggesting a connection between neutrophil "priming" and upregulation of HLA-DR. These data suggest that CL causes expansion of a subset of HLA-DR+ PMNs that are primed for activation.

1904

HLA DR EXPRESSING LOW DENSITY NEUTROPHIL SUBSETS EXPAND DURING HUMAN VISCERAL LEISHMANIASIS AND CAN CONTRIBUTE TO T CELL PROLIFERATION

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Visceral leishmaniasis (VL) is a chronic infectious parasitic disease, which if left untreated is almost always fatal.The role of neutrophils and how they affect or are affected during active VL is still unknown. Abebe et al reported that depletion of arginine was associated with suppressed T cell activity. We know that T cells from our subjects with visceral leishmaniasis respond differently to antigen when studied in isolated PBMCs or in whole blood, so we examined the hypothesis that there might be distinct subsets of circulating neutrophils in subjects with this infection. Indeed our studies revealed an enhanced population of low density neutrophils, similar to Abebe et al (2013 reference above).We fractionated whole blood over Ficoll to obtain both low density and normal density neutrophil populations. The neutrophils were identified and gated on Forward Side scatter and stained cells were CD66b+ CD14-, CD3-. The neutrophils were stained with HLA DR, CD80, CD86, CD63, CD11b, CD62L in our studies. For Neutrophil T cell cocultures we MACS purified CD66b cells and CD3 T cells from PBMCs and CD15 cells from NDG.In whole blood and fractions of whole blood i.e NDG and LDG we found that LDG were much more abundant during active disease and stained strongly for HLA DR. These cells were of different density and also expressed co-stimulatory molecules like CD80 and CD86. We performed Neutrophil-T cell co-culture experiments that lead us to this interesting finding that neutrophils can contribute to antigen presentation and proliferation in T cells. The CD66b+ neutrophils in VL subjects were CD62L low, CD11b high and are CD63 high which indicates that they are activated and de-granulating. This study was performed on 83 Active VL subjects (29 female and 54 male). Our data indicate there are indeed unusual neutrophil subsets that expand during visceral leishmaniasis, and underscore the need to further our understanding of neutrophil populations in infectious and inflammatory diseases.

1905

ACTIVATION OF HUMAN KERATINOCYTES BY *LEISHMANIA* SPP.: DIVERGENT EFFECTS OF *LEISHMANIA INFANTUM* VERSUS *LEISHMANIA MAJOR*

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All *Leishmania* infections are initiated in skin, but clinical manifestations vary greatly with infecting species. *Leishmania major* (*Lm*) causes localized cutaneous lesions, whereas *L. infantum* (*Li*) causes potentially fatal disseminated disease without skin pathology. Early responses at the skin infection site influence adaptive responses to *Leishmania*, yet little is known of the role of keratinocytes. We hypothesized that *Leishmania* induce keratinocytes to produce factors influencing the immune response. Incubation of *Li* with primary or immortalized human keratinocytes caused a significant increase in pro-inflammatory cytokine transcripts *il6*, *il8*, *tnfa,* and *il1b* measured by RT-qPCR. However, keratinocytes exposed to five distinct *Lm* isolates did not induce these transcripts, highlighting a species-specific difference in inflammatory response. Similar to live parasites, *Li*-derived exosomes induced more *il8* mRNA compared to control ($p<0.01$) or *Lm*-derived exosomes ($p<0.05$). Western blotting confirmed NFkB-p65 phosphorylation in keratinocytes exposed to *Li* but not *Lm*. To examine whether soluble keratinocyte factors influence nearby immune cells, *Li*-

infected human monocytes were co-cultured with keratinocytes across a trans well membrane. Soluble products of *Li*-exposed keratinocytes improved monocyte control of parasite replication compared with unexposed controls ($p<0.01$). However, culture with *Lm*-exposed keratinocytes across the trans well did not affect monocyte *Lm* infection. These data suggest that (1) activated keratinocytes may increase monocyte leishmanicidal activity and (2) keratinocytes support an early inflammatory environment, uniquely tailored to each *Leishmania* species, at the infection site.

1906

HEMOPHAGOCYTOSIS IN EXPERIMENTAL VISCERAL LEISHMANIASIS BY *LEISHMANIA DONOVANI*

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Visceral leishmaniasis (VL), also known as kala-azar, is caused by parasitic protozoa of the genus *Leishmania*. VL is characterized by clinical manifestations such as fever, weight loss, hepatosplenomegaly and anemia. Hemophagocytosis is a phenomenon of macrophages or histiocytes phagocytosing blood cells. There are reports on up-regulated hemophagocytosis in patients with infectious diseases including typhoid fever, tuberculosis, influenza and VL. However, mechanisms of infection-associated hemophagocytosis remained elusive due to lack of appropriate animal models. Here, we have established a mouse model of VL representing hemophagocytosis in order to elucidate mechanisms behind this phenomenon in VL. At 24 weeks after infection with 1 x 10^7 promastigotes of *Leishmania donovani*, BALB/cA mice exhibited splenomegaly with an average tissue weight of 10 times as those of naïve mice and anemia with lower hematocrit, hemoglobin and red blood cell counts than the naïve mice. In the spleen, 28.6% of macrophages contained erythrocytes. All of hemophagocytosing macrophages were parasitized by *L. donovani*. When hemophagocytes were categorized based on the number of parasites per macrophage, higher levels of hemophagocytosis were observed in heavily infected cells (more than 20 amastigotes). Besides, more than half of these hemophagocytes had two or more nuclei per cell whereas only 15.0% of splenic macrophages were multi-nucleated. Such multi-nucleated cells were not observed in spleens of uninfected mice. From these histological observations, hemophagocytes were presumed to be macrophages which acquired abnormal character by *L. donovani* infection. Through *in vitro* experiment with RAW264.7 cells, enhanced hemophagocytosis by macrophages was reproduced by infection with *L. donovani* in the presence of IFN-γ. These results suggested that *L. donovani* causes hyper-activation of macrophages to hemophagocytose. To our knowledge, this is the first report on hemophagocytosis in experimental *Leishmania* infections and may be useful to further understanding of the pathogenesis.

1907

INVOLVEMENT OF NUCLEOTIDE-BINDING DOMAIN LEUCINE-RICH REPEAT PROTEIN 12 (NLRP12) IN VISCERAL LEISHMANIASIS (VL)

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Leishmania infantum chagasi (Lic) causes VL, with suppression of type 1 immune responses. The NLR proteins include >20 cytosolic proteins that regulate inflammation and immunity. Activation of three NLRs or AIM2 can cause assembly of an inflammasome leading to IL-1β and IL-18 release. Functions of non-inflammasome forming NLRs are not as well understood. We hypothesized that NLR proteins influence the course of VL by modifying the localized inflammatory response to Lic. We screened for NLR effects by infecting NLR pathway gene knockout or wild type (WT) mice with Lic coexpressing luciferase and mCherry. Progressive parasite expansion was monitored by *in vivo* imaging (IVIS), qPCR and, Luciferase assay. The screens suggested involvement of the non-inflammasome forming Nlrp12 in progression of VL. Lic parasite loads expanded early (day 28) but were controlled in WT mice, whereas Lic continued to expand and were 2-fold higher than WT on day 56 of Nlrp12-/- infection. Consistently, liver-derived infiltrating cells from Nlrp12-/- mice released less antigen-induced IFNgamma than WT cells on infection day 56 (24 vs. 41 pg/mL). Flow Cytometry showed inflammatory monocytes expanded on day 28 in WT but not Nlrp12-/- mice, preceding parasite clearance from WT. Instead, resident macrophages expanded in Nlrp12-/- mice in parallel with the late expanding parasite load (day 56). The kinetics of monocyte derived dendritic cell (MNDC) recruitment paralleled parasite load, with recruitment at 28 days in WT but recruitment at 56 days in Nlrp12-/- mice. These data suggest that Nlrp12 plays a protective role in VL, associated with recruitment of both inflammatory monocytes and MNDCs at the time of peak parasite growth, followed by parasite clearance. Infiltration of inflammatory monocytes is impaired in the absence of Nlrp12, leading to delayed MNDC influx, impaired IFN-gamma, and expansion of resident macrophages, which permit parasite growth.

1908

EVALUATION OF THE USE OF *LEISHMANIA DONOVANI* DOUBLE KNOCK-OUT PARASITES (LDCEN-/-MIF-/-) AS PROTECTIVE VACCINE AGAINST VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is a neglected tropical disease, and fatal if untreated. There is no vaccine available against VL. Parasite persistence is thought to be important for an effective protective response. Such protection may also be achieved by immunization with gene-deleted live attenuated parasites that do not cause disease. We have previously reported on a genetically modified live attenuated parasite, with a cell division specific centrin1 gene deletion, producing strong immune protection in mice, hamsters and dogs. *Leishmania* parasites are shown to secrete an inflammatory cytokine, macrophage migration inhibitory factor (MIF) that causes poor T cell responses in the infected host due to excessive inflammation. In this study we have tested the *L. donovani* double gene deletion mutant strains deficient for centrin and MIF genes (LdCen-/-MIF-/-) for their safety and efficacy as a candidate vaccine. Our hypothesis is the double-attenuated strain induces more effective immune response through production of long-term memory T cells, being able to promptly respond to infection inducing protective response. Balb/c mice were immunized with LdCen-/-, LdMIF-/- or LdCen-/-MIF-/- parasites, and the immune responses were compared to a control group (PBS). After 4 weeks of immunization (4wpi), some mutant parasites were detected in spleen and liver by serial dilution. Our preliminary results showed that, at 4wpi, LdCen-/-MIF-/- immunized group presented higher percentage of CD4 and CD8 central memory T cells, higher percentage of CD8 late effector memory T cells, and increased CD8 T cells proliferation after specific stimulation compared to PBS and LdMIF-/-. Protective immunity induced by LdCen-/-MIF-/- parasites is currently being evaluated in mice by parasitological and immunological assays following 4 wpi, and 4, 8 and 12 weeks of challenge with wild type strain of L. infantum. These results demonstrate the role of parasite products involved in manipulating the host immunity and manipulating these mechanisms might enhance the vaccine induced protective immunity and help further development of vaccines against VL.

1909

TEST AND NOT TREAT (TNT): A SAFE STRATEGY TO PROVIDE COMMUNITY-BASED TREATMENT WITH IVERMECTIN IN *LOA LOA* ENDEMIC AREAS

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Implementation of ivermectin (IVM)-based community treatment for onchocerciasis or lymphatic filariasis (LF) control/elimination has been delayed in Central Africa because IVM can induce serious adverse effects (SAE) in people with *Loa loa* microfilaremia exceeding 30,000 microfilariae (mf)/mL blood. The recent development of CellScope-Loa, a rapid field-friendly diagnostic tool to quantify *L. loa* mf in peripheral blood, permits point-of-care (POC) identification of the few "at risk" individuals for exclusion from IVM treatment (to prevent SAEs) while the rest of the population can be safely treated. This "Test and not Treat" (TNT) strategy was evaluated in Okola district (Central Cameroon) where onchocerciasis and loiasis are co-endemic and where IVM distribution was halted in 1999, after the occurrence of SAEs including fatalities. Between August and October 2015, 16,205 individuals from a target population (>5 years) of 22,800 (participation: 71.1%) were tested at the point of care (POC) using the CellScope-Loa; those with fewer than a pre-determined threshold (20,000 mf/mL) were given IVM (n=15,469), whereas those above this threshold (n=343, 2.1%) were excluded from IVM treatment, in addition to 167 pregnant women and 226 people in a poor state of health). Adverse events were closely monitored by local volunteers and mobile medical teams visiting each village 1, 2, 3 and 6 days after treatment. No SAE was observed. A total of 970 individuals (6.3% of the IVM-treated population) experienced mild adverse effects (itching, rash, headache, arthralgia, myalgia, fever) that resolved within one week. About half of adverse events occurred in individuals who had no Loa mf before treatment. The TNT strategy based on the CellScope-Loa is an extremely promising and practical approach to the safe implementation of large-scale IVM-based treatment for LF and onchocerciasis elimination in Loa endemic areas.

1910

THE MACROFILARICIDAL ACTIVITY OF A SINGLE DOSE OF IVERMECTIN, ALBENDAZOLE AND DIETHYLCARBAMAZINE AGAINST *WUCHERERIA BANCROFTI* IN CÔTE D'IVOIRE

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Current single dose treatments for lymphatic Filariasis (LF) have limited ability to kill adult worms. In a recent pilot study in Papua New Guinea we showed a single dose of co-administered ivermectin (IVM, 200ug/kg), diethylcarbamazine (DEC, 6mg/kg), and albendazole (ALB, 400mg, IDA) completely cleared microfilaria (mg) 1 year after treatment compared to 8% clearance with DEC/ALB. However, the effect of IDA on macrofilaria is not known. We used ultrasounds of the spermatic cord and inguinal lymphatic vessels immediately prior to treatment and 6 months later to compare the effects of two drug regimens on adult filarial worms in infected men in Côte d'Ivoire. The first group included 46 men treated with a single dose of IVM+ALB (IA, mean number of worms nests=3.2±1.4 [range 1-13]) and the other group included 28 men who received IDA. Number of worm nests was the same at baseline (IA=3.2±1.4 range [1-13], IDA=3.0±1.4 [1-8]). Thirty-six men treated with IA and 21 men treated with IDA underwent repeat ultrasound after 6 months. Worm nests were cleared more often after IDA (15 of 21, 71%) than after IA (9 of 36, 25%, P=0.0009). IDA also showed a reduction in nest size of 83%, compared to 9% in the IA group, as well as 95.3% clearance of mf compared to 28.6% clearance of mf in IA (P<0.0001). These results suggest that a single dose of IDA killed most adult W. bancrofti and that IDA is more effective against adult filarial worms than IA.

1911

NEXT GENERATION IMMUNOASSAYS PROVIDE ONE-STEP SPECIES-SPECIFICITY FOR THE DIAGNOSIS OF FILARIAL INFECTIONS AND *STRONGYLOIDES STERCORALIS IN* TRAVELERS AND IMMIGRANTS

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Antifilarial antibody testing in the evaluation of returned travelers and immigrants to North America has relied on IgG- and IgG4-specific responses to crude filarial extracts (BmA). The anti-BmA IgG response is highly sensitive (~100%), but suffers from relatively poor (50%) specificity. It also suffers from significant cross-reactivity with *Strongyloides stercoralis* (Ss) and cannot distinguish among the infecting filarial species. Conversely, the IgG4 anti-BmA antibody test is close to 100% specific but has sensitivities that range between 50%-70%. Of the 10173 CLIA-certified antifilarial antibody tests performed, 1809 (18%) filarial infections were diagnosed based on a positive IgG4 anti-BmA antibody response, and 4908 (48%) were excluded using an IgG anti-BmA test below the defined cutoff. Over the same period, filarial- (Ov16, Wb123, LL-SXP1) and Ss (SsIR, Ss-NIE)-species-specific recombinants have been identified and characterized. Each of these, when configured in a variety of single antigen IgG4-based immunoassay formats have demonstrated close to 100% specificity for the species of interest but with variable sensitivities depending on the antigen. Thus, to create an all-in-one assay for screening of returned travelers and immigrants where infections with filariae or Ss

is being considered, we configured a multiplex suspension array assay to measure the IgG or IgG4 responses to BmA, LlSXP1, Ov16, Wb123, SsNIE and SsIR. When these multiplexed assays were assessed using serum samples from parasite-uninfected (n=70) subjects compare to definitively diagnosed (parasite-positive) infected patients with *Loa loa* (N=37), *Onchocerca volvulus* (n=185), *Wuchereria bancrofti* (N=24), and Ss (N=41) we were able to get IgG4 based assays that achieved 100% specificity for all infections and sensitivities that ranged from 67% for LL-SXP1 to 92% for Wb123. Using this novel multiplexed immunoassay, we have been able to de-convolute the anti-BMA reactivity and identify the species of infecting parasite responsible for the antibody positivity for better accuracy in the diagnosis of individual filarial and Ss infections.

1912

HIGH EFFICACY OF SINGLE DOSE OF CO-ADMINISTERED IVERMECTIN, DIETHYLCARBAMAZINE AND ALBENDAZOLE IN TREATMENT OF LYMPHATIC FILARIASIS IN CÔTE D'IVOIRE

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Available treatments for lymphatic filariasis (LF) are limited in their long-term clearance of microfilaria (mf) from the blood. Recently we reported that a single dose of co-administered Ivermectin (IVM, 200ug/kg), Diethylcarbamazine (DEC, 6mg/kg), and Albendazole (ALB, 400mg) completely cleared mf 1 year following treatment, compared to 8% clearance with DEC/ALB alone in a small pilot study in Papua New Guinea. In order to confirm and expand these results in a different population we performed an open-labeled, single-blinded clinical trial where microfilaremic infected individuals were randomized into those treated with IVM/DEC/ALB (IDA, N=42) or IVM/ALB (IA, N=55) and mf levels measured immediately prior to and 6 months following treatment. Of those enrolled 83% are men, with median age of 37 years and overall geometric mean mf of 191.4 mf/ml (range 51-2,250). In the IDA group 22 of 25 (88%) completely cleared their microfilaria. The remaining 3 each had a single mf per 2 ml of blood. By contrast only 32.4% of individuals treated with IA completely cleared their mf, with the remaining participants averaged 92.8% (range 58.2-98.6%) reduction in mf levels. Adverse events (AEs) particularly fevers, myalgias, and pruritus were common, occurring in 54.8% vs 40.4% of those receiving triple-drug compared to 2-drug treatment respectively (P=0.18); all symptoms resolved within 7 days after treatment. IDA had more level 2 (scale 1-3) reactions [9 (21%) vs 1 (2%) in IA], however no serious AEs were observed in either group. This confirms that triple-drug therapy is safe and more effective than IVM/ALB for Bancroftian filariasis and has the potential to accelerate elimination of lymphatic filariasis.

1913

EFFECTIVENESS AND SAFETY OF ALBENDAZOLE FOR THE TREATMENT OF HYPERMICROFILAREMIC LOIASIS IN GABON

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Loiasis is endemic in Gabon with prevalences ranging from 10 and 35 %. Ivermectin, the drug known to be associated with serious adverse reactions in case of *Loa loa* hypermicrofilaremia, is used for the prevention of lymphatic filariasis and onchocerchiasis in areas where they coexist with loiasis. Albendazole is an alternative to ivermectin. Its efficacy and safety for the treatment of loiasis microfilaraemia was assessed in 128 individuals with more than 8000mf/mL. Parasitological data and clinical symptoms were monitored at days 7, 14, 21 and 63 after administration of increasing doses of albendazole (50 or 100mg to 400mg and 800mg/day) during six to eight weeks. Asthenia was the predominent drug-related adverse event recorded. The percentage of participants with ≥ 50% decrease of microfilaraemia from pre-treatment to 1 month was 69%. At 3 months post-treatment, 82% of patients had no microfilaraemia detected after leuconcentration of 4mL blood. Objective symptoms were not noticed after 3 months and pruritus was the most frequently reported post-treatment clinical symptom. Data analysis is still ongoing. In conclusion, treatment of hypermicrofilaraemic loiasis with albendazole was safe and efficacious in continuously exposed patients.

1914

DEVELOPMENT OF MURINE MODELS OF LOIASIS TO ASSESS MICROFILARICIDAL ACTIVITY OF PRE-CLINICAL CANDIDATE ANTI-FILARIAL DRUGS

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Development of new macrofilaricides to eliminate onchocerciasis in Africa requires assessments of safety for potential indications in loiasis co-endemic regions. This is because rapid killing of *Loa loa* microfilariae (mf) following ivermectin (IVM) treatment in patients with high parasitaemias has been linked to the development of severe neurological adverse reactions. Via human pharmacokinetic profiling of IVM and the related macrocyclic lactone moxidectin (MOX) we define a mis-match between *in vitro* and *in vivo* drug sensitivities to the bloodborne human filariae *Brugia malayi* and *L. loa*. This indicates that safety evaluation of potential macrofilaricides requires screening *in vivo* against blood-stage *L. loa* mf. Here we describe the development of mouse models of loiasis with the goal of evaluating them as *in vivo* microfilaricide drug screens. BALB/c WT or SCID (+/- splenectomy) were perfused with Loa or Brugia mf. CB.17 SCID, NOD.SCID or NOD.SCID IL-2gc-/- (NSG) strains were infected with Loa L3 and evaluated at 3-5 months post-inoculation. Recovered worms were then surgically re-implanted in NSG mice and evaluated 1 month post-implantation. To evaluate drug responsiveness, microfilariaemic mice were treated with bio-equivalent IVM. The vast majority of perfused mf (~10% of initial inoculates) were sequestered in the cardiopulmonary circulation. Splenectomy increased both the incidence of peripheral Loa mf in WT mice and the overall yield of cardiopulmonary mf six days post infusion. IVM induced a rapid decline (>80%) in circulating mf in WT and SCID mice. For patent *Loa* infections, NSG mice yielded an average recovery of adult worms of 33% of the initial inoculate at +5 months. No circulating mf were observed although embryograms of female worms identified occurrence of embryogenesis and inter-uterine mf. For the adult

Loa implanted NSG mice, circulating mf were observed both centrally and peripherally. IVM treatment reduced microfilaraemia in these mice. In conclusion, preliminary validation demonstrates both models could be implemented as pre-clinical macrofilaricide counter-screens and are thus in further development.

1915

PET/CT LYMPHOSCINTIGRAPHY DEMONSTRATES EARLY CHANGES IN LYMPHATIC FUNCTION IN THE *BRUGIA MALAYI*/FERRET MODEL OF LYMPHATIC FILARIASIS

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The goal of this study was to evaluate changes in lymphatic function in the *Brugia malayi*/ferret model of lymphatic filariasis. Injection of L3 larvae into the ferret footpad results in intralymphatic infection of the femoral and inguinal lymphatics. Development of microfilaremia and eosinophilia begin at 12-14 weeks post-infection. Ferrets were imaged at baseline, and again at 2 and 16 weeks post-infection. Imaging was performed using a Simeons Inveon Multimodality PET/CT scanner. Anesthetized animals were subcutaneously injected (between the toes of their right hind limb) with 18F-FDG (100-150 uCi), and monitored for tracer uptake for 90 minutes. A CT scan for anatomical localization followed PET analysis. This approach enabled assessment of lymphatic function by quantification of tracer uptake into the inguinal lymph nodes over time. While single infectious challenges with L3s did not result in clinical disease as monitored by measurements of ankle and limb circumferences, they clearly caused marked changes in lymphatic anatomy and function as early as 2 weeks post infection. Compared to baseline imaging, expanded networks of tortuous and dilated lymphatic vessels were observed at all infected timepoints, along with the generation of collateral lymphatic vasculature. Whereas peak tracer uptake into the inguinal lymph nodes was observed by 20 minutes post-tracer injection in uninfected animals, peak uptake did not occur until 25-30 minutes after two weeks of infection and until 30-35 minutes after 16 weeks of infection. Additionally, maximal intensities of tracer signal in the inguinal lymph nodes was reduced by 50% in infected animals at all timepoints evaluated. These results demonstrate that *Brugia malayi* cause marked alterations in lymphatic vessel anatomy and function early in the course of LF infection; occuring prior to microfilaria production and in the absence of frank clinical disease. In current studies we are evaluating whether this imaging protocol can be used to assess alterations in lymphatic function induced by treatment with antifilarial agents.

1916

MICROBIOLOGY AND OUTCOMES IN HOSPITALIZED NEONATES WITH SEPSIS: A ZAMBIAN COHORT STUDY

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Sepsis is a major cause of mortality in neonates in sub-Saharan Africa, yet is not well-studied. We are conducting an ongoing cohort study of infants hospitalized in a large neonatal intensive care unit in a public hospital in Zambia. Clinical and demographic data were captured by maternal interview and chart review. Blood cultures were obtained on all neonates with suspected sepsis. We examined the microbiology and outcomes of neonates with all-cause bacteremia and those with *Klebsiella* infection. From September 1, 2015 to March 31, 2016, we enrolled 711 neonates, of whom 376 (53%) were male, and 543 (76%) were born at the study hospital. The median birth weight was 2690g (IQR 1600-3135g) and 539 (77%) were born vaginally without instrumentation. Most enrolled infants had suspected sepsis (n=520; 73%), of whom half (n=265; 51%) had a culture-confirmed bacteremia. The most common isolates were *K. pneumoniae* (n=187; 70%), common skin commensal organisms (n=55; 21%), and *Enterococcus* spp. (n=22; 8%). There was one isolate each of *Staphylococcus aureus* and *streptococcus agalactiae*. Overall mortality was 31% and was greater among bacteremic infants than septic, non-bacteremic infants (36% vs. 27%, p= 0.04). Among bacteremic neonates, low birth weight (aOR=1.02) and *Kleb.* infection (aOR=1.80) were independent risk factors for death. Most *Kleb.* isolates were resistant to fluoroquinolones (n=177, 95%) and half were resistant to β-lactam antibiotics, (n=93, 50%), with 29 (15%) ESBL. Mortality among neonates with *Kleb.* infections was 42%. The risk of death was similar among infants with β-lactam-resistant as compared to susceptible strains of *Kleb.* (44% vs. 41%, p= 0.77). Age at sepsis onset, maternal HIV status, and birth location were not associated with death in either the all-cause bacteremia or the *Kleb.*-infected cohorts. Neonatal sepsis was common, often caused by multi-drug resistant organisms, and associated with a high case-fatality rate in this large NICU in Zambia. *Kleb.* infection was associated with an increased risk of death; however, infection with β-lactam-resistant *Kleb.* was not associated with an increased risk of death.

1917

HIGH SERUM ZINC LEVELS PROTECT AGAINST ROTAVIRUS INFECTION BUT NOT OTHER DIARRHEA-ASSOCIATED PATHOGENS IN A BIRTH COHORT IN BANGLADESH

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Diarrhea remains the world's second leading cause of death in children. In combatting this challenge, zinc supplementation has been shown to reduce diarrheal morbidity and mortality, though the mechanisms are unclear. In most zinc efficacy trials, diarrhea has been treated as pathogen-agnostic; however rotavirus causes an estimated 40% of acute diarrhea in developing countries where rotavirus vaccines perform poorly and alternative methods for diarrheal management are critical. In the PROVIDE study, a randomized controlled trial of oral rotavirus vaccine in a birth cohort in Dhaka, Bangladesh, we found a significant protective effect of serum zinc concentration at age 18 weeks on the risk of rotavirus diarrhea through one year based on rotavirus antigen detection by ELISA in diarrheal stools. Using advanced molecular techniques, here we interrogate whether the protective effect of zinc is specific to rotavirus or extends to other diarrheal pathogens. We performed multiplex PCR on 1,448 diarrheal specimens collected between weeks 18-52 with the following targets: rotavirus, norovirus, sapovirus, astrovirus, Giardia, Cryptosporidium, and Entamoeba histolytica. Among these pathogens, only rotavirus correlated with zinc status (P=0.016, Kruskal-Wallis test). This relationship was further tested by logistic regression to include variables that may modify the effect of zinc: courses of zinc supplementation, sex, urinary mannitol at week 40, household food deficit, exclusive breast feeding and stunting. In the final model of risk of rotavirus diarrhea, children with zinc levels in the highest quartile compared to children at risk of zinc deficiency were nearly four times more likely to have diarrhea without any rotavirus detected versus rotavirus

infections (OR 3.93, 95% CI 1.28 - 12.05, P=0.017). Other factors in the model were not associated with rotavirus infection and did not significantly modify the effect of zinc. These results suggest a particular utility of zinc interventions in reducing the burden of rotavirus diarrhea compared to other etiologies. Future research will examine mechanisms of zinc protection in rotavirus.

1918

DECLINING CHILD MORTALITY DUE TO INFECTIOUS DISEASES IN AN URBAN SLUM IN NAIROBI KENYA

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Examining trends in causes of child mortality over time can help measure progress towards achieving Millennium Development Goal #4. Little is known about trends in child mortality in urban slums, where living conditions are precarious. We assessed child mortality rates and cause of death (COD) from 2009-2014 in a population of ~25,000 residents of Kibera slum in Nairobi, Kenya, under demographic surveillance. Participants were visited biweekly in their homes and queried about any deaths. Trained verbal autopsy (VA) interviewers used standardized World Health Organization VA questionnaires to gather data from a credible respondent on the circumstances leading up to death. InterVA-4 was used to code the most likely COD. We calculated rates of death per 1000 person-years-observation (pyo); for cause-specific rates, we extrapolated the observed proportion of COD by year to those with missing VA data. From 2009-2014, 336 deaths were reported among children aged <5 years. The child mortality rate declined from a high of 18.3/1000 pyo in 2009 to a low of 9.8/1000 pyo in 2013, then increased slightly to 11.4/1000 pyo in 2014. VA was completed on 255 (76%); 18 (7%) were classified as 'indeterminate' and 5 (2%) as 'other and unspecified neonatal'. Among the remaining 232 with a likely COD identified, the leading cause was acute respiratory infection/pneumonia, including 13/35 (37%) neonatal deaths and 102/197 (52%) in children aged 29 days to <5 years. Other common causes included malaria (n=29, 12%), diarrhea (n=12, 5%), and HIV/AIDS-related (n=12, 5%). Rates of pneumonia deaths peaked in 2010 at 9.8/1000 pyo, then fell to a low of 4.5/1000 pyo in 2014. Rates of all other infectious causes combined (excluding pneumonia) decreased from 9.8/1000 pyo in 2009 to 2.9/1000 pyo in 2013 and 2014. We observed a reduction in child mortality of more than 35% in recent years in Kibera. The decline was driven by falling mortality due to pneumonia and other infectious diseases. Possible factors contributing to improved survival include introduction of the pneumococcal conjugate vaccine in 2011 and scaled up malaria control and HIV prevention efforts.

1919

A SYSTEMATIC REVIEW ON THE EFFECTIVENESS OF STRATEGIES TO IMPROVE HEALTH WORKER PERFORMANCE IN LOW- AND MIDDLE-INCOME COUNTRIES: PRELIMINARY RESULTS ON UTILIZATION OF HEALTH SERVICES

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Improving health worker (HW) performance is a global health priority. Strategies that improve performance might also increase utilization of health services. To characterize the effectiveness of such strategies in low- and middle-income countries (LMICs), we conducted a systematic review of 15 electronic databases, 30 document inventories of international organizations, and bibliographies of 510 articles. We included studies meeting accepted criteria for methodological adequacy (e.g., trials with comparison groups) of any strategy to improve HW performance on any health topic in any language, published or not. This analysis focuses on studies that measured continuous outcomes on the utilization of health services (e.g., number of patients seen per month). Effect sizes were calculated as percent change over time in the intervention group minus percent change over time among controls. We screened >105,000 citations, and 822 reports met inclusion criteria. Fifty-seven studies measured continuous utilization outcomes and were included in the analysis. Many strategies have been tested, usually with multiple intervention components. However, most strategies were tested by only one study. The median effect size (MES) across all studies was an improvement of 15 percentage-points (%-points) (interquartile range [IQR]: -14, 57). Among studies of facility-based HWs, three strategies tended to increase utilization: strategies that included financial incentives for HWs or health facilities (MES = 67 %-points, IQR: 2, 119), insurance schemes (MES = 16 %-points, IQR: -44, 47), and reducing or removing user fees (MES = 15 %-points, IQR: -12, 42). Introducing or increasing user fees tended to decrease utilization (MES = -53 %-points, IQR: -82, -17). Among studies of lay HWs, no clear patterns were identified. For example, strategies that included the combination of HW training + supervision + patient or community education had effect sizes of -29, 5, 79, and 306 %-points. Contextual and methodological heterogeneity made comparisons difficult. These results should inform decision-making on increasing utilization of health services in LMICs.

1920

THE IMPACT OF ANEMIA DURING PREGNANCY AND ITS RISK FACTORS ON THE COGNITIVE DEVELOPMENT OF ONE-YEAR-OLD CHILDREN

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The aim was to investigate the impact of anemia during pregnancy and its risk factors on the cognitive development of one-year-old children. Our prospective cohort study included 636 mother-singleton child pairs from 828 eligible pregnant women who were enrolled during their first antenatal care visit (ANV) in Allada, Benin, into the MiPPAD clinical trial. Venous blood samples of women were assessed for ferritin and hemoglobin (Hb) concentrations at the first and second ANV of at least one-month interval and at delivery. Stool samples of pregnant women were also collected during these follow-up periods to test for helminths using the Kato-Katz technique. All pregnant women were administered a total of 600 mg of mebendazole (100 mg two times daily for 3 days) to be taken after the first ANV. Women were also given daily iron and folic acid supplements throughout pregnancy. The intake was not directly observed. At age one year, cognitive and motor functions of children were assessed using the Mullen Scales of Early Learning. The prevalence of iron deficiency (ID) among pregnant women at first and second ANC visits, and at delivery was 30.5%, 34.0% and 28.4%, respectively. Prevalence of helminth infection was 11.5%, 7.5% and 3.0% at first, second ANV and at delivery, respectively. Prevalence of anemia decreased from 67.1% at first ANV [mean gestational age (Standard deviation), 22.1(4.0) weeks] to 40.1% at delivery. Children of mothers who were infected with hookworms at first ANV had 4.9 (95% confidence interval, CI: 1.3 - 8.6) lower mean gross motor scores compared to those whose mothers were not infected with hookworms at the first ANV. We observed a significant negative quadratic relationship between infant gross motor function and Hb concentration at first and second ANVs. Prenatal helminth infection is associated with poor with infant cognitive and motor development. However, in the presence of iron supplementation, ID is not associated with infant neurocognitive development. Further, there appears to be an Hb concentration range (90-110 g/L) that may be optimal for better gross motor function of one-year-old children.

1921

MATERNAL AND INFANT FACTORS MEDIATING COGNITIVE DEVELOPMENT AT 12 MONTHS AMONG FILIPINO INFANTS

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The objective of this study was to identify pre- and post-natal predictors that directly or indirectly affect infant cognitive, language, and motor development at 12 months of age among Filipino infants and the pathways through which they act. The Bayley Scales of Infant Development, 3rd edition, was used to assess the development of 314 infants who were enrolled in a trial to examine the effects of Praziquantel for the treatment of schistosomiasis given at 12-16 weeks gestation on pregnancy outcomes. Covariates evaluated from the trial included maternal iron, socio-economic, and nutritional status, as well as birthweight and newborn iron status. Infant nutritional status, iron and hemoglobin were captured at 1, 6, and 12 months of age and the Philippines Nonverbal Intelligence Test (PNIT) was administered to mothers. Multivariable linear regression and structural equation modeling were used to identify significant factors associated with infant development. In multivariable regression models, maternal treatment with Praziquantel, education, PNIT score, and iron status as well as infant WAZ, WLZ, and WAZ gains were significantly associated with specific domains of infant development at 12 months of age. Structural equation models demonstrated that maternal PNIT scores [standardized β (sβ) for cognitive=0.073, sβ for language=0.061, sβ for motor=0.20, all P <0.05] directly influenced most subscales of infant development and indirectly impacted development through birthweight and/or infant weight gain. Maternal iron status during gestation was a stronger predictor of development than infant iron status. Infant change in nutritional status was related to language and motor development (eg, sβs of WAZ gain/mo for language=0.15 and motor=0.079, all P <0.05), suggesting catch up growth may ameliorate some cognitive deficits among LBW infants. Finally, exclusive breast feeding had a direct effect on infant expressive language, rather than through improved iron or nutritional status. This study identifies modifiable risk factors for impaired infant development beginning *in utero* and the key pathways through which they act.

1922

MOTHERS SCREENING FOR MALNUTRITION BY MUAC IS NON-INFERIOR TO COMMUNITY HEALTH WORKERS: RESULTS FROM A LARGE-SCALE PRAGMATIC TRIAL IN RURAL NIGER

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Community health workers (CHWs) commonly screen for acute malnutrition in the community by assessing mid-upper arm circumference (MUAC) on children aged 6-59 months. MUAC is a simple screening tool that has been shown to be a better predictor of mortality in acutely malnourished children than other practicable anthropometric indicators. This study compared, under program conditions, mothers and CHWs in screening for severe acute malnutrition (SAM) with color-banded MUAC tapes and checking for edema. It took place from May 2013 to April 2014 in two health zones of Niger's Mirriah District. Mothers in Dogo (Mothers zone) were trained to screen children for malnutrition in their household and CHWs in Takieta (CHWs zone) were trained to screen children in the community. Exhaustive coverage surveys were conducted quarterly, and relevant data collected routinely in the health and nutrition program. An efficacy and cost analysis of each screening strategy was performed. 12,893 mothers were trained in the mothers zone and 36 CHWs in the CHWs zone, and point coverage was similar in both zones at the end of the study (35% [26/74] Mothers Zone vs 32% [11/34] CHWs zone; p=0.7772).The rate of MUAC agreement (compared with health center agent) was higher in the Mothers zone (75.4% [721/956] vs 40.1% [221/551]; p<0.0001) and cases were detected earlier, with median MUAC at admission for those enrolled by MUAC <115 mm estimated to be 1.56 mm (95%CI 0.65-1.87) higher using a smoothed bootstrap procedure. Children in the mothers zone were less likely to need inpatient care, both at admission and during treatment, with the most pronounced difference at admission for those enrolled by MUAC <115 mm (0.7% [4/569] vs 7.8% [32/413]; risk ratio 0.09 [95%CI 0.03-0.25]; p<0.0001). Training mothers required higher up-front costs, but overall costs were much lower ($8,600 USD vs $21,980 USD). Mothers were not inferior to CHWs in screening for malnutrition at a substantially lower cost, and children in the Mothers zone were admitted at an earlier stage of SAM with fewer hospitalizations. Empowering mothers to screen for malnutrition should be a part of treatment programs globally.

1923

INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN HIV-INFECTED PREGNANT WOMEN WITH DIHYDROARTEMISININ-PIPERAQUINE: A DOUBLE BLINDED RANDOMIZED CONTROLLED TRIAL

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Intermittent preventive therapy with sulfadoxine-pyrimethamine (IPT-SP) is recommended for the prevention of malaria among HIV-uninfected pregnant women in sub-Saharan Africa. The WHO recommends that HIV-infected pregnant women receiving daily trimethoprim-sulfamethoxazole (TS) prophylaxis should not be given SP due to drug toxicity concerns. Although daily TS has been shown to be more effective than IPT-SP, resistance to this class of antimalarials is widespread, especially in East Africa. We recently showed that IPT with dihydroartemisinin-piperaquine (DP) was more effective than SP for the prevention of malaria in pregnancy in HIV-uninfected women. To extend this approach to HIV-infected women, we are conducting a double blind randomized placebo controlled trial comparing daily TS alone with daily TS plus monthly DP in Tororo District, Uganda. 200 HIV-infected pregnant women between 12-28 weeks gestational age were enrolled between December 2014 and October 2015. At enrollment, all women received a long lasting insecticide treated bed net and ensured to be taking combination antiretroviral therapy. Participants are being followed in a dedicated study clinic for all their medical care and encouraged to deliver at an adjacent hospital.The primary outcome is the risk of placental malaria defined by histopathology. Secondary outcomes include placental malaria defined by placental blood smear, birth outcomes, and the incidence of adverse events. As of 29th February 2016, 188 women had delivered and 12 were still being followed during pregnancy. The risk of placental malaria was 6.4% by histopathology and 0.6% by placental blood smear. Adverse birth outcomes include 3 spontaneous abortions (1.6%),1 stillbirth (0.5%), 5 congenital anomalies (2.7%),16preterm deliveries (8.7%), and 23 with low birth weight (12.4%). It is anticipated that all women will have delivered by April 2016 and that the final un-blinded results of the trial will be presented at the meeting.

1924

IMPACT OF EFAVIRENZ AND PREGNANCY ON PIPERAQUINE EXPOSURE IN UGANDAN PREGNANT WOMEN

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In malaria endemic areas HIV+ pregnant women receiving EFV-based combination antiretroviral therapy (EFV-cART) may receive artemisinin-based combination therapies (ACTs) for the treatment or prevention of malaria. One ACT, dihydroartemisinin-piperaquine (DHA-PQ), has shown excellent efficacy for the treatment of falciparum malaria and for intermittent preventive therapy (IPT) in pregnancy. We evaluated PQ pharmacokinetics in the setting of DHA-PQ and EFV-cART in pregnant (28 wks gestation) and postpartum Ugandan women using an intensive design. These studies were included as part of our trials (PROMOTE) in Tororo, Uganda to inform IPT dosing guidelines. PQ levels after standard dosing of DHA-PQ (qD x 3d) were compared between a) HIV- (no cART, n=30) and HIV+ (EFV-cART, n=26) pregnant women to determine the impact of EFV and b) HIV - antepartum (n=30) and postpartum (n=23) women to determine the impact of pregnancy. The area under the concentration-time curve (AUC) was measured over 21 d. PQ levels were measured by LC tandem MS. We found highly significant decreases in PQ exposure for HIV+ women on EFV compared to HIV- women, as measured by AUC (6.60 vs 10.6 hr.ug/mL; GMR:0.62, p<0.005) and Day 7,14, and 21 PQ levels (5.46, 1.62, 0.668 ng/mL vs 15.5, 5.37, 3.78 ng/mL; GMR:0.35, 0.30, and 0.18, respectively, all p values <0.0001). Pregnancy was also associated with decreased PQ AUC when comparing ante-partum and post-partum women (10.6 vs 17.2 hr.ug/mL; GMR:0.63, p<0.0001), and day 7, 14, and 21 PQ concentrations (15.5, 5.37, 3.78 ng/mL vs 32.8, 17.4, 11.4 ng/mL; GMR: 0.47, 0.31, 0.33, respectively, all p values ≤0.0001). EFV and pregnancy resulted in significant reductions in PQ exposure. For both HIV+ and HIV- pregnant women, mean PQ terminal concentrations were consistently <10 ng/mL, lower than the concentration previously estimated to be required for effective chemoprevention (30 ng/mL). Clinical correlates of these findings are underway. DHA-PQ dose escalation for pregnant women and those receiving EFV-cART may merit study.

1925

MALARIA IN HIV-INFECTED CHILDREN RECEIVING HIV PROTEASE-INHIBITOR-COMPARED WITH NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR-BASED ANTIRETROVIRAL THERAPY

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HIV and malaria geographically overlap. HIV protease inhibitors kill malaria parasites *in vitro* and *in vivo*, but further evaluation in clinical studies is needed. Children from Malawi, Uganda, and Zambia were enrolled in P1068s, a substudy within a larger randomized HIV treatment study. Children aged 4-36 months were followed every 3 months and at intercurrent illness visits for up to 47 months between September 2009 and December 2011. We compared malaria parasite carriage by blood smear microscopy (BS) and confirmed clinical malaria incidence (CCM, or positive BS with malaria symptoms) in children initiated on HIV antiretroviral therapy (ART) with zidovudine, lamivudine, and either nevirapine (NVP), a non-nucleoside reverse transcriptase inhibitor, or lopinavir-ritonavir (LPV-rtv), a protease inhibitor. Because of low rates of BS positivity at Ugandan and Zambian sites, we analyzed results from Malawi only, an area of low to moderate malaria transmission intensity. We found an association between increased time to recurrent positive BS, but not CCM, when anti-malarial treatment and LPV-rtv based ART were used concurrently and when accounting for a LPV-rtv and antimalarial treatment interaction (adjusted HR 0·39; 95% CI (0·17,0·89); p=0·03). In our study, LPV-rtv in combination with malaria treatment is associated with lower risk of recurrent positive BS, but not CCM, in HIV-infected children. Larger, randomized studies are needed to confirm these findings which may permit ART optimization for malaria-endemic settings.

1926

EFFECT OF DAILY TRIMETHOPRIM SULFAMETHOXAZOLE PROPHYLAXIS ON THE LONG-TERM CLINICAL IMPACT OF MALARIA INFECTION AMONG HIV INFECTED ADULTS ON SUCCESSFUL ART IN BLANTYRE, MALAWI

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Sub-Saharan Africa has 90% and 70% of all new cases of malaria and HIV respectively. The risk of malaria infection is higher in HIV infected adults. Malaria infection in HIV positive individuals is associated with increased HIV plasma viral load (VL) and decreased CD4+ T cells. Daily trimethoprim sulfamethoxazole (TS) reduces the risk of malaria infection in HIV positive individuals but its long term benefit after successful ART has not been well documented. To determine the impact of TS on malaria infection and disease, we analyzed data from clinically stable, non-pregnant HIV infected adults on non protease inhibitor ART enrolled in an ongoing randomized controlled trial in Blantyre, an area with low to moderate malaria transmission. Participants with CD4 count >250 cells/mm3 and HIV VL of <400 copies/ml were enrolled and randomized to continue daily TS, discontinue TS, or discontinue TS and begin chloroquine. During the rainy season, we measured asymptomatic infection by quantitative PCR of dried blood spots. Clinical malaria was diagnosed in participants with symptoms suggestive of malaria and positive malaria smear by microscopy. We included only a subset of participants who continued on TS prophylaxis (n=34) or stopped prophylaxis (n=27). The two groups were similar in age, gender distribution, CD4 count, hemoglobin level and bed net use. Four participants in the TS discontinuation group developed clinical malaria compared to only one from the daily TS group. No episodes of asymptomatic malaria infection were detected by qPCR. Even in this lower transmission setting, TS prophylaxis was associated with protection against clinical malaria disease. The absence of asymptomatic malaria infection is in contrast with the common finding of high rates of low-level asymptomatic parasitemia in Malawi. HIV infected adults may be more likely to develop symptomatic disease; another possible interpretation is that ART or malaria prophylaxis confer some protection. We are currently undertaking immunological evaluation to determine mechanism of this observed phenomenon.

1927

PERSISTENCE OF LOWER ANTIBODY LEVELS TO VAR2CSA IN HIV-POSITIVE KENYAN PREGNANT WOMEN DESPITE HAART

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Malaria and HIV epidemics intersect in sub-Saharan Africa, disproportionally affecting young women, including those of childbearing age. Pregnancy provides *Plasmodium falciparum* parasite an additional niche for evading the immune system. Parasite-infected erythrocytes sequester in the placenta using the VAR2CSA adhesion molecule, causing placental malaria. Antibodies (Ab) against VAR2CSA improve pregnancy outcomes and a vaccine based on VAR2CSA is under clinical evaluation. Ab levels to VAR2CSA are lower in HIV-positive women compared to healthy controls. It is not clear whether widespread HAART implementation and immune reconstitution in HIV-positive pregnant women will improve their Ab responses to VAR2CSA. In a longitudinal case-control study we compared Ab levels to the full—length VAR2CSA (FV2) and its individual DBL domains (DBL1-DBL6), antibody avidity and cytokine levels between HIV-positive pregnant women receiving HAART and HIV-negative Kenyan pregnant women. At delivery no significant differences were observed in peripheral plasma levels of IL1β, IL2, IL4, IL6, IL7, IL8, IL10, IL12, IL21, IFNγ, TNFα, MIP1α, MIP1β between HIV-positive and HIV-negative women (all p>0.05) after adjusting for malaria status (PCR) and gravidity. In a multiple regression model adjusted for malaria status and gravidity, HIV was associated with significantly lower Ab levels at delivery to FV2, DBL1+2, DBL3 and DBL5 (all FCR3 strain); no significant differences were observed for DBL2, DBL4 and DBL6. In addition, HIV was associated with 5% decrease of Ab avidity to FV2 (p=0.03). Ab data from earlier visits during pregnancy are currently being analyzed. Lower Ab levels at delivery in HIV-positive women could contribute to less protection from placental malaria during the next pregnancy. Data on Ab responses to VAR2CSA in HIV-positive women on HAART are important in order to guide VAR2CSA-based vaccine regiments for HIV-positive women in sub-Saharan Africa.

1928

ASSESSING IMPACT OF COMMUNITY-BASED ANTIRETROVIRAL THERAPY AND ITS SCALE UP: PERSPECTIVES FROM FOUR PRIORITY LOCAL GOVERNMENT AREAS IN LAGOS, NIGERIA

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Community-based antiretroviral therapy (cART) delivery is effective in improving the early identification of HIV-positive clients, access to treatment and quality of health outcomes of people living with HIV. The cART project scale-up (cART Plus) aimed to further improve on those gains in achieving more individual testing in the community, improve ART coverage, strengthen linkage and retention in care and subsequently achieve viral suppression over the long-term; in line with the UNAIDS 90-90-90 targets. The cART project is being supported by USAID through SIDHAS. 4 LGAs in Lagos Nigeria were selected based on epidemiological and mapping indices. Community volunteers were recruited and trained in areas subsequently working with the CBOs to carry out community mobilization, household testing and counselling, identify positives and enrol to care, track and follow up on defaulters, documentation and reporting of service output data using approved tools. Data analysis spanning October to December 2015 in these 4 LGAs showed that the indices assessed significantly improved upon cART scale-up (cART Plus). Number of individuals counselled, tested and received results for HIV in the community {cART (Oct: 24 307); cART Plus (Nov: 61 532; Dec: 50 049)}. Number of individuals tested HIV-positive {cART (Oct: 65); cART Plus (Nov: 364; Dec: 268)}. The positivity rate in the general population being {cART (Oct: 0.27%); cART Plus (Nov: 0.59%; Dec: 0.54%)}. Number of persons newly enrolled into the ART programme for PreART care in the community {cART (Oct: 34); cART Plus (Nov: 283; Dec: 214)}. Percentage enrolment {cART (Oct: 52.3%; cART Plus (Nov: 77.7%; Dec: 79.9%)}. Number of persons newly started on ART in the community {cART (Oct: 13; cART Plus (Nov: 106; Dec: 123)}. In conclusion, the cART delivery scale-up has shown to improve uptake and accessibility of treatment. This concept could be adopted in more resource-limited settings to improve ART coverage. However, efforts need to be channeled into advocacy for community ownership as community programs need to be driven, owned by and embedded in the communities.

1929

DEVELOPMENT OF A MUCOSAL VACCINE AGAINST HIV BASED ON GENETICALLY-ENGINEERED *SACCHAROMYCES CEREVISIAE* PROBIOTIC STRAINS

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Human immunodeficiency virus (HIV) is a major public health problem. It is estimated that 37 million people worldwide are infected with HIV and 2 million new infections are reported each year. A vaccine against HIV is urgently required to stop this epidemic. An efficient prophylactic vaccine strategy must induce a mucosal immune response, since most infections occur during sexual intercourse through vaginal and rectal mucosae. Probiotic Saccharomyces cerevisiae strains are known to provide health benefits in the gut when administered in correct doses, including stimulation of secretion in the colon. We engineered several probiotic *S. cerevisiae* strains to express the HIV GAG antigen on their surface, and are assessing the GAG expression levels and the genetically-engineered strains ability to resist gastrointestinal stresses. We are also quantifying the *in vitro* phagocytosis rates of GAG-expressing yeasts by macrophages and quantifying the levels of tumor necrosis factor-alpha (TNF-α), interferon-gamma, (IFN-γ), interleukin (IL)-1β, IL-5, IL-6, IL-8, IL-10, and IL-12 secreted by these antigen presenting cells following contact with the fungal engineered vectors. We are currently developing a humanized mouse model to evaluate the efficacy of genetically-modified S. cerevisiae probiotic strains as a potential prophylactic vaccine against HIV.

1930

DIALOGUE BETWEEN NEUTROPHILS AND HOOKWORMS DETERMINES PARASITE DEVELOPMENT

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Hookworms are skin-penetrating parasites infecting about 700 million people, principally within improvised communities. The skin has recently been shown to be an important bulwark against parasite establishment in immune hosts. However, the initial interaction between the host and parasite within the skin following the first encounter with the parasite is still poorly characterized. Here, we investigate the fate of the larvae from their skin penetration to their migration to the lungs using intravital microscopy. We observe that neutrophils are rapidly recruited to the site of infection and adhere to the larvae. Surprisingly however, neutrophils are not sufficient to cause parasite killing. We further show that the parasite adjusts its development to the presence of the neutrophils by an evasion demarche: on one hand, the parasite delays its exsheathment to benefit from an additional layer of cuticle protection; on the other, in response to bleach induced by the neutrophils, the parasite secretes specific Excretory-Secretory (ES) products with anti-neutrophil activity. Building on these observations, we show that vaccination with parasitic ES products renders the parasite susceptible to killing by neutrophils, presumably by allowing the host to neutralization parasitic products capable of interfering with neutrophil activity. Altogether, this study highlights that targeting both the nematode's sensing mechanism and its secretory products with neutrophil inhibitory potential could enable parasite killing early during its migration and thus block its transmission.

1931

GENOMIC ABLATION OF CYST-WALL-PROTEIN-1 PREVENTS STAGE-SPECIFIC FORMATION OF GOLGI-LIKE ORGANELLES AND REGULATED SECRETION OF A CYST WALL IN *G. LAMBLIA*

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The genome of the ubiquitous protozoan parasite G. lamblia is organized in two diploid nuclei, which has so far precluded complete analysis of gene function. Here we used a previously developed Cre-loxPbased knock-out and selection marker salvage strategy in the human-derived isolate WB-C6 to eliminate all four copies of the Cyst Wall Protein 1 locus (cwp1). Because the cwp1 loci are silenced in proliferating trophozoites and expressed only in encysting cells, complete CWP1 ablation allowed functional characterization of a conditional phenotype in differentiating cells. Induced -cwp1 cells show morphological hallmarks of cyst development as well as karyokinesis, but are unable to establish the stage-regulated trafficking machinery with Golgi-like encystation-specific vesicles required for cyst wall formation. The wall-less "pseudocyst" phenotype could be rescued by transfection with an episomallymaintained CWP1 expression vector. This is the first example of genome editing and functional analysis or a locus essential for transmission between hosts in a diplomonad parasitic species.

1932

ADIPOSE TISSUE IS A MAJOR RESERVOIR OF FUNCTIONALLY DISTINCT *TRYPANOSOMA BRUCEI* PARASITES

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In the mammalian host, *Trypanosoma brucei* parasites are thought to reside mainly in the blood. Although these parasites are also present in the interstitial spaces of organs, such as brain, the extent of these extravascular sites has never been assessed. Using a mouse model, in this study, we identified the adipose tissue as a previously unknown major reservoir of *T. brucei* parasites. Histology and quantitative studies revealed that, in chronic stages of disease, there are 100-fold more parasites in adipose tissue than blood and 800-fold more than brain. Morphometric analysis of a GFP::$PAD1_{UTR}$ reporter cell-line showed that adipose tissue parasites (ATFs) can be found as slender, intermediate and stumpy forms. We also showed that ATFs are capable of infecting naïve mice, suggesting they are viable and can reestablish a blood infection. To test if parasites from adipose tissue and blood are functionally different, we performed RNA-seq of these parasites. ATFs are remarkably distinct from their blood counterparts in several key regulatory processes, including putative fatty acid –oxidation enzymes. Pulse-chase biochemical assays confirmed that ATFs are indeed able to catabolize exogenous myristate and form –oxidation intermediates, suggesting that ATF parasites can use fatty acids as an external carbon source, a behavior never previously reported for any life cycle stage of this parasite. All together, these findings identify the adipose tissue as a niche for *T. brucei* during its mammalian life cycle. In the future, it will be interesting to test if this is the cause of the weight loss associated with sleeping sickness and to investigate how such a large parasite reservoir impacts population dynamics and transmission to other hosts.

1933

ANALYZING THE CRYPTIC STATOR OF THE ATP SYNTHASE COMPLEX IN *TOXOPLASMA GONDII*

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The mitochondrial F_o-F_1 ATP synthase is a macromolecular complex present in almost every organism that couples the proton-motive force generated by respiration to synthesis of ATP. The complex can be divided in two main portions: F_1, which has the catalytic sites for ATP synthesis; and F_o, which forms a channel allowing protons to move down their electrochemical gradient. The F_o portion of the ATP synthase also contains a stator, which is needed to resist the rotational torque of F_1. In apicomplexans, little is known about the organization and function of the ATP synthase. While all the F_1 constituents have been identified, the information about the F_o subunits is fragmentary, and sequence-based searches have failed to identify any stator subunits. By performing a genome-wide CRISPR-based screen in *Toxoplasma gondii*, we identified several mitochondrially-localized proteins, unique to apicomplexans, and essential for survival in human fibroblasts. One such subunit had structural similarity to the ATP synthase b subunit, a central stator component. Tagging the protein endogenously showed that it is localized to the parasite mitochondria and that it co-immunoprecipitates with all the ATP synthase F_1 subunits, consistent with its putative role as the b subunit. Visualized by negative stain electron microscopy, the complex assumes the typical organization, as well as unusual higher-order arrangements. We are currently studying the function of the putative stator through a series of genetic and biochemical approaches. The study of the cryptic apicomplexan stator will yield new knowledge about the function of the ATP synthase in these parasites, and uncover potential therapeutic susceptibilities.

1934

TOXOPLASMA GONDII INTERACTIONS WITH THE HOST LIPID DROPLETS: RECRUITMENT, NEUTRAL LIPID SCAVENGING AND CONSEQUENCES

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Toxoplasma gondii has evolved to recruit host mammalian organelles to its parasitophorous vacuole (PV) in part to divert essential nutrients present in host organelles. We explored the role of host lipid droplets (LD) as sources of neutral lipids for the parasite. We demonstrate that host LD cluster around the PV and LD numbers increase and then decrease with infection, suggesting that *Toxoplasma* manipulates these structures. Indeed, *Toxoplasma* scavenges lipids from host LD, in part through the interception of Rab7-associated LD, and through the translocation of intact host LD into its PV. In mammalian cells, the exogenous addition of oleic acid (OA) up to 1mM is non-toxic and stimulates LD biogenesis. When exposed to 0.2mM OA, intravacuolar *Toxoplasma* profusely scavenges OA, channels this fatty acid to newly formed LD, with a concurrent increase in transcriptional activities of neutral lipid-generating enzymes However, this condition slows down both parasite replication and egress. By comparison, 0.2mM palmitic acid added in the medium does not affect parasite development whereas 1mM jasmonic acid boosts parasite growth. Our ultrastructural analyses of OA-loaded *Toxoplasma* reveal, for the first time, the presence of coated pits at the parasite's plasma membrane and additional structures potentially involved in endocytosis. More dramatically, exogenous addition of 0.4 mM OA results in the massive accumulation of lipid deposits in the PV and within parasite organelles, leading to replication defects and death. This highlights the high sensitivity of *Toxoplasma* towards deleterious effects of accumulating OA. Deciphering the lipotoxic response of the parasite may reveal new vulnerabilities amenable to controlling *Toxoplasma* infections.<!--EndFragment-->

1935

IDENTIFICATION OF BROADLY CONSERVED CROSS-SPECIES PROTECTIVE *LEISHMANIA* ANTIGEN AND ITS RESPONDING CD4$^+$ T CELLS

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Despite a plethora of publication on immunology of leishmaniasis, there is still no vaccine against the disease. Recovery from natural or experimental infection with *Leishmania major* induces long-term protection to reinfection collectively known as infection-induced resistance. However, it is not known what antigens induce and maintain this resistance and whether these antigens preferentially favor the development of memory T cells. To identify protective *Leishmania* antigens, we eluted and identified naturally processed *L. major* peptides from I-A^b MHC II molecules on infected BMDCs by immunoproteomics approach. One of the peptides activated *Leishmania*-reactive T cells from mice that have healed their primary *L. major* infection *in vitro*. Interestingly, the source protein of this peptide, glycosomal phosphoenolpyruvate carboxykinase (PEPCK), was expressed in both the promastigote and amastigote stages of the parasite. Also, cellular immune responses against PEPCK were detected in *L. major*-infected patients, while antibody responses were detected in infected mice, dogs and human. I-A^b-PEPCK$_{335\text{-}351}$ tetramer identified for the first time protective Leishmania-specific CD4$^+$ T cells at clonal level, which comprised ~ 20% of all Leishmania-reactive CD4$^+$ T cells at the peak of infection. PEPCK$_{335\text{-}351}$-specific CD4$^+$ T cells are oligoclonal in their TCR usage, produce polyfunctional cytokines (IL-2, IFN-- and TNF) and undergo expansion, effector activities, contraction and stable maintenance following lesion resolution. Vaccination with PEPCK peptide, DNA expressing full length PEPCK or rPEPCK induced strong durable cross-species protection in both resistant and susceptible mice. Given the remarkable effectiveness and durability of protection in vaccinated mice, our study suggests a real possibility for development of a broadly cross-species protective vaccine against different forms leishmaniasis by targeting PEPCK.

1936

A NOVEL POPULATION OF NATURAL KILLER CELLS PLAYS A CRITICAL ROLE IN THE DEPLETION OF SPLENIC B2 B CELLS DURING EXPERIMENTAL AFRICAN TRYPANOSOMIASIS

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Mice infected with Trypanosoma brucei, the causative agent of human sleeping sickness and a contributor to nagana in cattle, rapidly lose the capacity to mount VSG-specific antibody responses, and die with uncontrolled parasitemia. We have shown (Bockstal et al., 2011, PLOS Pathogens) that the loss of humoral immune competence in the infected mice results from depletion of developing and mature splenic B cells. We now report that T. brucei-induced splenic B cell depletion is dependent upon the presence of the pore forming molecule perforin which is present in the cytotoxic granules of cytotoxic T lymphocytes, natural killer T cells and natural killer cells, occurs in the absence of T cells (and natural killer T cells), i.e., in T cell receptor ($\alpha\beta\gamma\delta$)-/- mice, but does not occur in intact mice that are depleted of natural killer (NK) cells by treatment with monoclonal antibody specific for the NK1.1 differentiation antigen. In the intact mice, B cells are deleted after remission of the first T. brucei parasitemic wave. At this time natural killer cells are expressing the cytotoxic granule marker CD107a, indicating that they have degranulated, executing their effector function. Moreover, in vitro assays show that

B cells from T. *brucei* infected mice are killed by natural killer cells from uninfected C57BL/6 mice but not efficiently killed by CD107a positive natural killer cells isolated from infected mice, which may be functionally exhausted.

1937

ENDOGENOUS PHOSPHOLIPASE A2 GROUP 1B (PLA2G1B) HAS DIRECT ANTI-HELMINTH PROPERTIES AND IS ESSENTIAL FOR IMMUNITY TO *HELIGMOSOMOIDES POLYGYRUS*

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With emerging evidence of drug-resistant helminths, it is important to identify mechanisms of anti-helminth immunity to provide new avenues of therapeutic intervention. To identify novel mechanisms of immunity we compared the small intestinal transcriptome of mice that were susceptible (primary infected, *H. p.* 1°) or resistant (secondary challenge infected, *H. p.* 2°) to the evolutionally adapted murine intestinal helminth *Heligmosomoides polygyrus*. We identified distinct clusters of genes in resistant mice, some of which have previously been described, and many that have not. In particular, we identified elevated expression of lipid metabolism pathways and the lipid catabolising enzyme, Phospholipase A_2 Group 1B (*Pla2g1b*), in resistant, but not susceptible mice. Elevated expression of *Pla2g1b* was dependent upon drug-mediated killing of *H. polygyrus* and was restricted to epithelial cells of the small intestine. Importantly, elevated expression of *Pla2g1b* was critical for immunity to *H. polygyrus*, as *Pla2g1b*$^{-/-}$ mice failed to expel a challenge infection with *H. polygyrus*. Proficient immunity to *Nippostrongylus brasiliensis*, but not *Trichuris muris,* also required *Pla2g1b* suggesting preferential requirement for *Pla2g1b* in the small intestine, but not large intestine. The failure to expel *H. polygyrus* in *Pla2g1b*$^{-/-}$ mice was not due to an ineffectual or aberrant immune response. Instead we show that Phospholipase A_2 Group 1B had a direct effect on *H. polygyrus* larvae, with *in vitro* treatment of L3 larvae compromising their ability to establish *in vivo* and treatment of *H. polygyrus* larvae restoring immunity in *Pla2g1b*$^{-/-}$ mice. Together, these data indicate that endogenous epithelial cell-associated *Pla2g1b* is required for direct killing of invading larvae; revealing a previously unrecognised *Pla2g1b*-dependent mechanism of anti-helminth immunity.

1938

A SERUM FACTOR REGULATES SEXUAL COMMITMENT IN P. FALCIPARUM

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Sexual commitment initiates production of the transmission-competent gametocyte stage in malaria parasites. Chromatin remodeling events at the *ap2-g* locus and activation of the encoded transcription factor are the earliest steps known in this differentiation process. Interestingly, the rate of gametocyte formation is not fixed and variation is thought to depend on environmental cues.

Using an assay to probe gametocyte formation *in vitro*, we found that parasites induce sexual commitment in response to depletion of human serum components. Fractionation experiments identified lysophosphatidylcholine (LysoPC) - a major phospholipid component of serum - as the active host factor. Low micromolar concentrations of LysoPC are sufficient to prevent sexual differentiation in *P. falciparum* cultured under otherwise commitment-inducing conditions. Metabolic labeling experiments revealed that parasites readily use LysoPC as a substrate for the synthesis of other lipids, including phosphatidylcholine (PC). In the absence of LysoPC, parasites induce a switch in lipid metabolism that is accompanied by the transcriptional up-regulation of enzymes used for *de novo* synthesis of PC (Kennedy pathway). RNAseq data revealed that LysoPC-depletion further induces the expression of both known (i.e. *ap2-g*) and new markers that define the transcriptional signature of commitment. A subset of those markers, including several kinases and cell cycle regulators, are currently under investigation. Altogether, our results provide unprecedented insights on how malaria parasites integrate external stimuli into the decision-making process of sexual differentiation.

1939

MOLECULAR DISSECTION OF *CRYPTOSPORIDIUM* LIFECYCLE

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Cryptosporidium is a leading cause of diarrhea and an important contributor to infant mortality. Neither efficacious drugs nor vaccines are available and our knowledge of the Cryptosporidium biology to drive their development is scant. Cryptosporidium has a single-host lifecycle, and completes its asexual and sexual phases in the same host. While one model suggests that Cryptosporidium sustains continued infection of a single host through an asexual cycle, we believe progression from asexual to sexual stages to be obligatory, and favor a developmental model of continued autoinfection with sporozoites. Sex is thus a requirement of chronicity and an important target of therapy. To unravel this process at the molecular level we have developed a series of transgenic C. parvum strains that mark different stages of the lifecycle with fluorescent reporters. Using these tools we demonstrate and define asexual and sexual stages in tissue culture and infected animals and we observe mating. These strains also allow us to enrich specific stages by flow cytometry to discover sets of genes uniquely expressed at different points of the parasite's lifecycle. ApiAP2 transcription factors are key regulators of apicomplexan development making them ideal targets to disrupt lifecycle progression. We have identified and tagged ApiAP2 factors that are expressed exclusively in early trophozoites, schizonts and female gamonts of Cryptosporidium parvum, respectively. Our current work uses conditional ablation of key AP2 genes to dissect the cellular mechanisms and transcriptional regulation of sex in Cryptosporidium.

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